(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number
WO 2005/014008 A2

(51) International Patent Classification[7]: A61K 31/70

(21) International Application Number:
PCT/US2004/022913

(22) International Filing Date: 16 July 2004 (16.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/488,339 17 July 2003 (17.07.2003) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/488,339 (CON)
Filed on 17 July 2003 (17.07.2003)

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ORTALDO, John** [US/US]; 5694 Glenrock Drive, Frederick, MD 21703 (US). **WILTROUT, Robert** [US/US]; 303 Cooper Oaks Drive, Woodboro, MD 21798 (US).

(74) Agents: **PATTON, Stephana, E.** et al.; Edwards & Angell, LLP, 101 Federal Street, P.O. Box 55874, Boston, MA 02205-5874 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/014008 A2

(54) Title: METHODS AND COMPOSITIONS FOR TREATING DISEASES AND DISORDERS ASSOCIATED WITH NATURAL KILLER T CELLS

(57) Abstract: The invention relates to methods and compositions useful in the treatment of autoimmune disorders or diseases as well as disorders or diseases having an autoimmune component such as AIDS. Preferred methods and compositions of the invention comprise use of a Beta Galactosyl Ceramide compound to treat a mammal suffering from or susceptible to such disorders. Methods and compositions of the invention can reduce NKT cells effectively *in vivo* in the substantial absence of undesired bystander events (cellular activation, cytokine production, systemic inflammation, etc). Methods and compositions of the invention can also reduce tumor size or ability to metastasize.

PATENT APPLICATION
Docket No.: 58172-PCT(47992) (E-282-2002/0-PCT-02)
EXPRESS MAIL LABEL NO.: EV 438970663 US

METHODS AND COMPOSITIONS FOR TREATING DISEASES AND DISORDERS ASSOCIATED WITH NATURAL KILLER T CELLS

This application claims priority to U.S. Provisional Patent Application Serial No. 60/488,339, filed July 17, 2003, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Research supporting this application was carried out by the United States of America as represented by the Secretary, Department of Health and Human Services.

FIELD OF THE INVENTION

The invention relates to methods and compositions useful in the treatment of autoimmune disorders or diseases as well as disorders or diseases having an autoimmune component such as AIDS, to reducing tumor masses of proliferative disorders, and to promoting successful organ and tissue transplants. Preferred methods and compositions of the invention comprise use of a beta Galactosyl Ceramide compound to treat a mammal suffering from or susceptible to such disorders.

BACKGROUND

Natural killer T (NKT) cells represent a small lymphocyte subpopulation that has been reported to have dramatic and important regulatory functions. NKT cells are characterized by their co-expression of the natural killer (NK) receptors NK1.1 or NKR-P1A (CD161) and a T-cell receptor (TCR). However, these NKT lymphocytes exhibit heterogeneity in both phenotypic characteristics and their functions.

NKT cells have recently been categorized into several distinct groups based on their T cell receptor (TcR) repertoire, expression of antigen, ability to recognize antigen presenting co-receptor molecules and their anatomical compartmentalization

in the host. Type I NKT is the T-cell population that has a rearrangement of the Vα14-Jα18 variable region of the T cell receptor and is either CD4+ or double negative (CD4-, CD8-). The responsiveness of these NKT cells are CD1d restricted and are primarily located in the thymus, liver, spleen and bone marrow. Reactivity appears restricted to alpha Galactosyl Ceramide (αGalCer) and represents a major component of the overall NKT cell population. This NKT type also co-expresses other NK receptors, e.g. Ly49 receptors. A second type of NKT cells is CD1d autoreactive and co-expresses a more distinct TcR expression pattern (Vα3.2-J9/Vα8, Vα8). Like Type 1, Type 2 NKT cells are CD4+ or double negative and express predominately NK1.1, however, they differ from Type 1 cells in their location in vivo and their antigen reactivity (nonreactive with αGalCer). A third type of NKT cells are not CD1d dependent, has a diverse TcR expression and can be either CD4+, CD8+ or double negative. Their antigen reactivity is not known and their in vivo distribution is similar to that of group 1 NKT cells. A fourth type of NKT cells is defined by their expression of α2-integrin (CD49B), recognized by the NK reactive antibody, DX5. This NKT population has little overlap with Type 1NKT cells and may have a very specialized role in the immune response.

Many of the studies that have examined NKT cells have centered on the role of the Type 1 and Type 2 - NKT cell as these populations represent a majority of spleen, liver and bone marrow associated NKT cells. These studies have utilized the reactivity of αGalCer to eliminate these cells and thus evaluate their role in immune regulation. Recently, a number of studies have demonstrated that the administration of αGalCer, in vivo results in the rapid depletion of NKT cells through a process that largely involves the induction of apoptosis through the TcR stimulation. A bystander effect of this NKT activation is the production of IFNγ and the activation of NK cells that often are found in the spleen and liver, resulting in systemic inflammation.

NKT cells have been shown to be involved in autoimmune disease and immune anergy to antigen stimulation. More studies have demonstrated in a number of model systems that NKT cells can regulate autoimmunity and often reverse the adverse effects of immune dysregulation. The application of these models to human disease may provide an avenue for treatment of autoimmune diseases and to increase

immuno-reactivity of cancer and AIDS patients to tumors and/or vaccines. To date, the only efficient means to remove NKT cells in vivo has been the use of KRN7000 (αGalCer). However, this reagent has been shown to exhibit potent bystander activation of NK cells and strong induction of cytokines in the host, resulting in the side effects of systemic inflammation, particularly in the spleen and liver. As such, αGalCer is not desirable as a therapeutic agent. Compounds which could act to reduce the numbers of NKT cells involved in such conditions as autoimmunity and AIDS (a viral disease with autoimmune components), while not inducing the production of cytokines and systemic inflammation, would be highly useful as therapeutic agents.

It thus would be desirable to have new methods and compositions to modulate NKT cells in a subject. It would be particularly desirable to have new methods and compositions that could modulate NKT cell populations without associated increased cytokine production.

SUMMARY OF THE INVENTION

We have now discovered therapies to treat or prevent various diseases and disorders involving Natural Killer T Cells (NKT Cells), including autoimmune disorders and disorders having an autoimmune component as well as promoting successful organ and tissue transplants. In particular, we have discovered therapies which can deplete a subject's NKT cell population without concomitantly increasing the subject's production of cytokines.

Preferred methods and compositions of the invention comprise the use of one or more beta Galactosyl Ceramide compounds to remove or otherwise reduce a population of NKT cells effectively *in vivo*, particularly in the substantial absence of deleterious side effects caused by the concomitant bystander events (including cellular activation, cytokine production, systemic inflammation, etc) that are found with the use of alpha Galactosyl Ceramides.

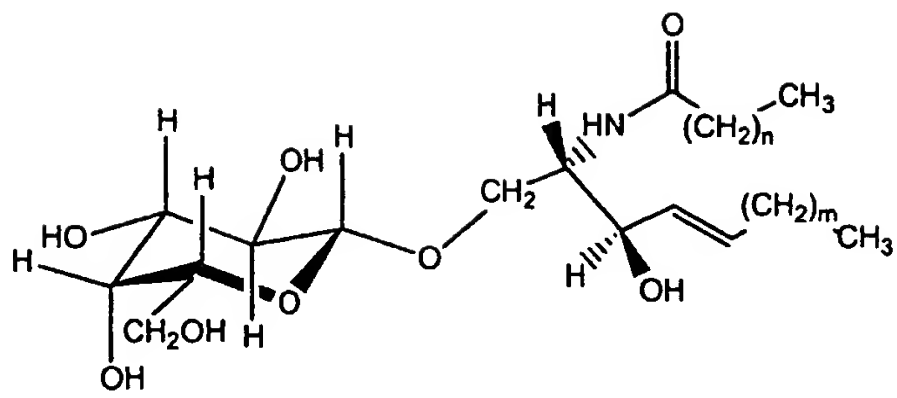
Preferably, an administered beta Galactosyl Ceramide compound does not cause a significant increase in production of cytokines. In any event, preferred administered beta Galactosyl Ceramide compounds will cause a lower production of

cytokines relative to that which would result from the administration of alpha Galactosyl Ceramides. Preferably, an administered beta Galactosyl Ceramide compound results in no more about 50%, more preferably no more than about 40, 30, 20 or 10 percent or even about a zero percent increase in cytokine production relative to production resulting from administration of alpha Galactosyl Ceramide. Cytokine production can be measured as described herein under Materials and Methods and used in Examples 6 and 7.

Suitable and preferred beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention can be readily identified through simple testing. For instance, suitable beta Galactosyl Ceramide compounds can be evaluated and identified by an *in vitro* assay of depletion of NKT cells (obtained from mammalian liver) as exemplified in Example 4 which follows, which in general includes contacting liver tissue with a candidate suitable beta Galactosyl Ceramide compound at room temperature and assessing the NKT cells population of the sample versus a control (same liver cells but not treated with any therapeutic agents) after 24 and 48 after administration. A candidate compound may be considered suitable for use in the methods and compositions of the invention if it provides a discernable decrease in NKT cell population relative to the control sample in either or both the assessment periods, e.g. At least about a 5, 10, 15 or 20 percent decrease in NKT cell amounts relative to the control. References herein to a standard *in vitro* NKT assay refers to that protocol, as also exemplified in Example 4 which follows.

Typical beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention will comprise a beta-configuration galactosyl moiety, suitably optionally substituted at one or more galactosyl ring positions by any of a variety of non-hydrogen substituents such as optionally substituted alkyl; optionally substituted alkyenyl; optionally substituted alkynyl; optionally substituted heteroalkyl; optionally substituted carbocyclic aryl; optionally substituted aralkyl; optionally substituted heteroalicyclic; optionally substituted heteroaryl; and the like.

Particularly preferred beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention include those of the following Formula I:



wherein n is an integer of from 5 to 20, preferably 8 to 15; and m is an integer of from 5 to 30, preferably from 10 to 20; and pharmaceutically acceptable salts or solvates thereof. A preferred compound of Formula I is D-Galactosyl-β1-1'-N-Dodecanoyl-D-erythroSphingosine.

A beta Galactosyl Ceramide compound may be administered or formulated in a composition in conjunction with one or more other agents, including a CD1d peptide, a nucleic acid sequence coding for a CD1d peptide and/or a CD1d-expressing cell, or other therapeutic agent such as agents known for use in autoimmune therapies.

Preferred therapeutic methods of the invention in general comprise administering an effective amount of one or more beta Galactosyl Ceramide compounds to a subject (e.g., a mammal particularly human) or cells (e.g. Mammalian cells particularly human in need thereof), e.g. A subject or cells that are suffering from or susceptible to a disease or disorder associated with NKT cells and/or autoimmune diseases and disorders such as, systemic lupus erythematosus, multiple sclerosis, diabetes mellitus and rheumatoid arthritis, or disease and disorders with autoimmune components such as Acquired Immunodeficiency Syndrome (AIDS) and treatment of persons suffering from or susceptible to HIV infection as well as treatment of a subject undergoing or having undergone a transplant procedure such as a tissue (e.g. Skin) or organ (e.g. heart, lung, kidney) transplant.

Therapeutic methods of the invention also include decreasing a population of mammalian particularly human Natural Killer T cells comprising administering one or more beta Galactosyl Ceramide compounds to cells or a subject.

Preferred therapeutic methods also may including identifying and/or selecting a subject or cells (e.g. A mammal or mammalian cells, particularly a human or human cells) that are susceptible to or suffering from a condition disclosed herein, and thereafter administering to the identified and/or selected subject or cells an effective amount of a β-Galactosyl Ceramide compound. Such identification and selection can be accomplished by any number of means, e.g. Testing a subject or cells for an autoimmune disease or disorder.

In a further aspect, the invention also provides use of a beta Galactosyl Ceramide compound for the treatment or prevention (including prophylactic treatment) of a disease or condition as disclosed herein, including autoimmune diseases and disorders such as systemic lupus erythematosus, multiple sclerosis, diabetes mellitus and rheumatoid arthritis, or disease and disorders with autoimmune components such as Acquired Immunodeficiency Syndrome (AIDS) and HIV infections as well as treatment of a subject undergoing or having undergone a transplant procedure.

In a yet further aspect, the invention provides use of a beta Galactosyl Ceramide compound for the preparation of a medicament for the treatment or prevention (including prophylactic treatment) of a disease or condition as disclosed herein, including autoimmune diseases and disorders such as systemic lupus erythematosus, multiple sclerosis, diabetes mellitus and rheumatoid arthritis, or disease and disorders with autoimmune components such as Acquired Immunodeficiency Syndrome (AIDS) and HIV infections as well as treatment of a subject undergoing or having undergone a transplant procedure.

In another aspect, methods to treat proliferative disorders, especially, liver metastasis are provided, including administering to a subject in need thereof one or more beta Galactosyl Ceramide compounds. In a related aspect, one or more beta Galactosyl Ceramide compounds are administered in conjunction with cytokines, preferably, IL2 to treat cancer. Other agents, such as other anti-proliferative compounds may also be administered.

The invention also includes pharmaceutical compositions that comprise a beta Galactosyl Ceramide compound optionally admixed with a pharmaceutically acceptable carrier and optionally packaged together with instructions (e.g. written) for use of the composition for a condition as disclosed herein.

Other aspects of the invention are described *infra*.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 graphically depicts effects of alpha galactosyl ceramide on various cell functions *in vivo* on mouse liver lymphocytes, wherein Panel A represents NK activity and is expressed in lytic units; Panel B depicts NK cells based on flow forward scatter; Panel C measures CD69 expression on NK cells (NK1.1+, CD3-); Panel D represents liver NK cell numbers; Panel E depicts NKT cells numbers; Panel F represents relative percent NK cells in liver; Panel G depicts relative percent NKT (NK1.1+, CD3+) cells in liver; Panel H represents total leukocyte number in liver and Panel I measures serum IFNγ levels in serum.

Figure 2 depicts a graphic representation of the effects of αGalCer on NK cell size and cell lysis in SCID mice.

Figure 3 depicts data graphs illustrating the comparison of αGalCer and βGalCer (C12), wherein Panel a represents NK cell percentage ; Panel B represents NK cytolytic activity in lytic units at 20% ; Panel C represents CD69 expression on NK cells; and Panel D represents percentage of NKT cells.

Figure 4 depicts various bar graphs illustrating that the administration of αGalCer but not β-GalCer (C12) results in a systemic activation of cells that produce cytokines.

Figure 5 depicts data graphs illustrating the comparative effects of α GalCer and β GalCer on serum cytokines and NKT early events in cell death.

Figure 6 depicts data graphs illustrating the binding of CD1d with of αGalCer and β-GalCer (C12).

Figure 7 depicts data graphs illustrating he effects of CD1d-mediated induction of cytokines in NKT and NK cells.

Figure 8 depicts data graphs illustrating the effects of αGalCer and β-GalCer (C12) on cytokine production from liver lymphocytes.

Figure 9 depicts data graphs illustrating the comparative effects of α GalCer and β GalCer on *in vivo* graft rejection of allogeneic bone marrow cells

Figure 10 depicts a data graph illustrating the decrease in renal cancer metastases upon administration of IL2 and β-GalCer.

Figure 11 depicts a nucleotide sequences encoding the CD1d polypeptide (SEQ ID NO:1).

Figure 12 depicts a CD1d polypeptide of SEQ ID NO:2.

DETAILED DESCRIPTION OF THE INVENTION

We now provide new therapeutic agents and methods that can decrease the population of number of NKT cells effectively *in vivo* . Preferred methods and compositions of the invention can provide such decrease of NKT cell populations in the significant absence of bystander events (cellular activation, cytokine production, systemic inflammation, etc.).

I. Beta Galactosyl Ceramide compounds:

As discussed above, suitable and preferred beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention may be identified by simple testing, including the *in vitro* NKT cell depletion assay exemplified in Example 4 which follows.

As also discussed above, typical beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention will comprise a beta-configuration galactosyl moiety, suitably optionally substituted at one or more galactosyl ring positions by any of a variety of non-hydrogen substituents such as optionally substituted alkyl; optionally substituted alkyenyl; optionally substituted alkynyl; optionally substituted heteroalkyl such as alkoxy, thioalkyl, aminoalkyl, sulfinylalkyl, sulfonylalkyl and the like; optionally substituted carbocyclic aryl preferably optionally substituted phenyl and naphthyl; optionally substituted aralkyl; optionally substituted heteroalicyclic; optionally substituted heteroaryl; etc.

For such beta Galactosyl Ceramide compounds, suitable alkyl substituent groups typically have from 1 to about 12 carbon atoms, more preferably 1 to about 8 carbon atoms, still more preferably 1, 2, 3, 4, 5, or 6 carbon atoms. As used herein, the term alkyl unless otherwise modified refers to both cyclic and noncyclic as well as branched and straight groups, although of course cyclic groups will comprise at least three carbon ring members. Preferred alkenyl and alkynyl groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention have one or more unsaturated linkages and typically from 2 to about 12 carbon atoms, more preferably 2 to about 8 carbon atoms, still more preferably 2, 3, 4, 5, or 6 carbon atoms. The terms alkenyl and alkynyl as used herein refer to both cyclic and noncyclic groups, although straight or branched noncyclic groups are generally more preferred. Preferred alkoxy groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention include groups having one or more oxygen linkages and from 1 to about 8 carbon atoms, and still more preferably 1, 2, 3, 4, 5 or 6 carbon atoms. Preferred alkylamino groups include those groups having one or more primary, secondary and/or tertiary amine groups, and from 1 to about 8 carbon atoms, still more preferably 1, 2, 3, 4, 5, or 6 carbon atoms. Preferred alkylsulfinyl groups having one or more sulfur atom linages and from 1 to 12 carbon atoms. Preferred alkylsulfinyl groups have one or more sulfinyl (SO) groups and from 1 to 12 carbon atoms. Preferred alkylsulfonyl groups have one or more sulfonyl (SO_2) and from 1 to 12 carbon atoms.

Suitable heteroalicyclic groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention contain one or more N, O or S

atoms and include, e.g., tetrahydrofuranyl, thienyl, piperidinyl, morpholino and pyrrolidinyl groups.

Suitable heteroaromatic groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention are 5-membered or 6-membered single ring moieties having at least N, O or S rings atoms. Particular examples of heteroaromatic groups include optionally substituted pyridyl, pyrrolyl, furyl (furanyl), thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadia-zolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl,1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, quinolizinyl, quinazolinyl, pthalazinyl, quinoxalinyl, cinnolinyl, napthyridinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5,6,7,8-tetrahydroisoquinolyl, purinyl, pteridinyl, carbazolyl, xanthenyl or benzoquinolyl.

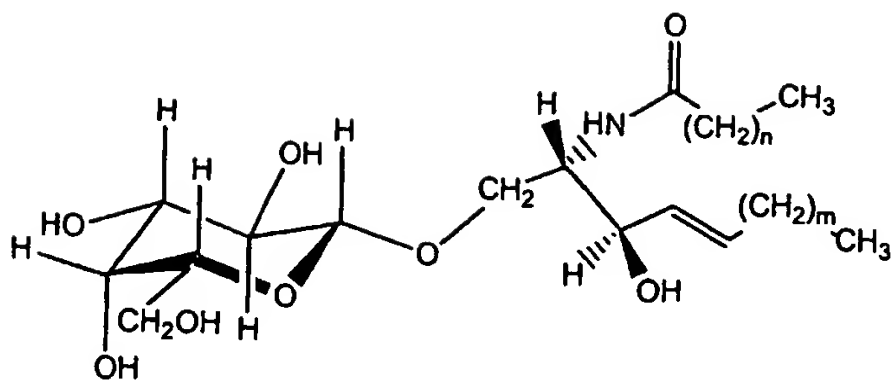
Suitable carbocyclic aryl groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical carbocyclic aryl groups of compounds of the invention contain 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms. Specifically preferred carbocyclic aryl groups include phenyl; naphthyl including phenyl, 1-naphthyl and 2-naphthyl; biphenyl; phenanthryl; anthracyl; and acenaphthyl. Substituted carbocyclic groups are particularly suitable including substituted phenyl, such as 2-substituted phenyl, 3-substituted phenyl, 4-substituted phenyl, 2,3-substituted phenyl, 2,4-substituted phenyl, and 2,5-substituted phenyl; and substituted naphthyl, including naphthyl substituted at the 5, 6 and/or 7 positions.

Suitable aralkyl groups of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused carbocyclic aryl groups. Typical aralkyl groups contain 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms. Preferred aralkyl groups include benzyl

and naphthylmethyl (-CH_2-naphthyl), and other carbocyclic aralkyl groups, as discussed above.

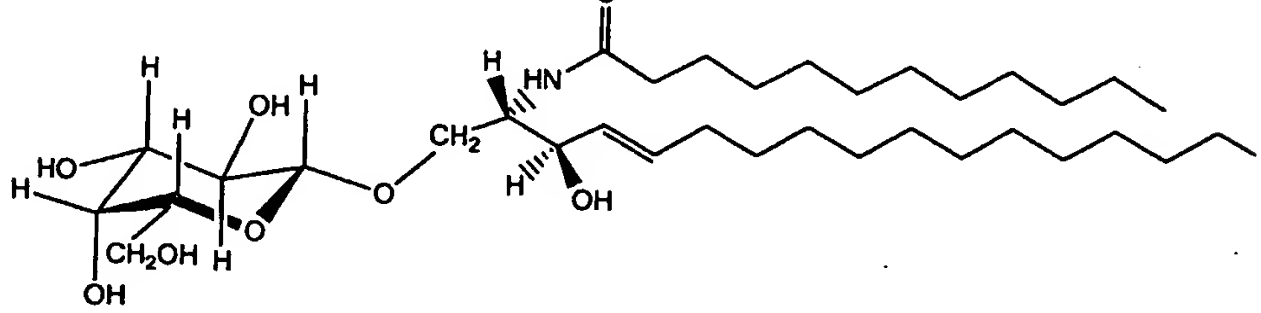
As discussed above, various substituents of beta Galactosyl Ceramide compounds for use in the methods and compositions of the invention may be optionally substituted. A "substituted"group or other substituent may be substituted by other than hydrogen at one or more available positions, typically 1 to 3 or 4 positions, by one or more suitable groups such as those disclosed herein. Suitable groups that may be present on a "substituted" group or other substituent include e.g. halogen such as fluoro, chloro, bromo and iodo; cyano; hydroxyl; nitro; azido; alkanoyl such as a C_{1-6} alkanoyl group such as acyl and the like; carboxamido; alkyl groups including those groups having 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to about 12 carbon, or 2, 3, 4, 5 or 6 carbon atoms; alkoxy groups including those having one or more oxygen linkages and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylamino groups such as groups having one or more N atoms and from 1 to about 12 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; carbocyclic aryl having 6 or more carbons; aralkyl having 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, with benzyl being a preferred group; or aralkoxy having 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, such as O-benzyl. Also comprised by the term optionally substituted shall be situations where at one position (atom) of a moiety two substituents (e.g. Two alkyl groups) undergo ring closure to provide e.g. For a cycloalkyl, such as a cyclopropyl, moiety.

Particularly preferred beta Galactosyl Ceramide compounds for pharmaceutical compositions and methods of use of the invention include those of the following Formula I:



wherein n = 8 to 15 and m = 10 to 20.

In a preferred embodiment, the present invention provides for a pharmaceutical composition comprising D-Galactosyl-β1-1'-N-Dodecanoyl-D-erythroSphingosine with a pharmaceutically acceptable carrier. This beta Galactosyl Ceramide has the following physical and chemical characteristics: Molecular weight : 643; Molecular Formula: $C_{36}H_{69}NO_8$ and Percent Composition: C 67.15%, H 10.80%, N 2.18% O 19.88% (Avanti Product Number: 860544).



D-Galactosyl-β1-1'-N-Dodecanoyl-D-erythroSphingosine

Other specific beta Galactosyl Ceramide compounds for pharmaceutical compositions and methods of use of the invention include D-Galactosyl-β1-1'-N-Nonanoyl-D-erthroSphingosine, D-Galactosyl-β1-1'-N-Decanoyl-D-erthroSphingosine, D-Galactosyl-β1-1'-N-Ondecanoyl-D-erthroSphingosine, D-Galactosyl-β1-1'-N-Propdecanoyl-D-erthroSphingosine, D-Galactosyl-β1-1'-N-Butdecanoyl-D-erthroSphingosine, and D-Galactosyl-β1-1'-N-Pentdecanoyl-D-erthroSphingosine.

Beta Galactosyl Ceramide compounds useful in the methods and compositions of the invention are commercially available from a number sources (e.g., Avanti Polar Lipids, Inc., Alabaster, AL, www.avanti.com; Kirin Pharmaceutical Research and Development, Gunma, Japan, www.kirin.co.jp.)

Beta Galactosyl Ceramide compounds useful in the methods of the invention also may be readily prepared by known synthetic procedures. For instance, an available compound such as D-Galactosyl-β1-1'-N-Dodecanoyl-D-erythroSphingosine may be modified (e.g. Substitution reactions) to include desired substituent groups.

II. CD1d Polypeptides and Nucleic Acids:

CD1 molecules are antigen presenting molecules that are able to bind and present glycolipids. These polypeptides are expressed on dendritic cells, monocytes and some thymocytes and function in antigen presentation to T cells. Without being bound by any theory, it is believed that CD1d polypeptides can bind or otherwise associate with a beta Galactosyl Ceramide compound and act to present the thus complexed a beta Galactosyl Ceramide compound as antigens specifically to NKT cells. In doing so, the interaction with NKT cells results in the induction of NKT apoptosis (i.e., cell death) but not in the NKT cell activation of cytokine production and release.

As discussed above, the invention provides for the use of one or more beta Galactosyl Ceramide compounds administered in coordination with one or more CD1d polypeptide, a nucleic acid sequence coding for a CD1 peptide and/or CD1d expressing cells. An isolated CD1d polypeptide is characterized as having essentially the amino acid sequence of SEQ ID NO:2. The term "isolated" as used herein refers to CD1d which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify CD1d polypeptide using standard techniques for protein purification. The isolated polypeptide will yield a single band on a non-reducing polyacrylamide gel. The purity of the CD1d polypeptide can also be determined by amino-terminal amino acid sequence analysis. CD1d protein includes functional fragments of the polypeptide, as long as the activity of CD1d, such as the ability to bind and present glycolipids

remains. Fragments or smaller peptides containing the biological activity of CD1d in terms of presenting glycolipids are included in the invention.

The invention provides for the use of nucleotide sequences encoding the CD1d polypeptide (SEQ ID NO:1) for purposes of producing CD1d polypeptides and CD1d expressing cells. These nucleotides include DNA, cDNA, and RNA sequences which encode CD1d. It is also understood that all nucleotide sequences encoding all or a portion of CD1d are also included herein, as long as they encode a polypeptide with CD1d activity. Such nucleotide sequences include synthetic, and intentionally manipulated nucleotide sequences. For example, CD1d nucleotide sequences may be subjected to site-directed mutagenesis. The nucleotide sequences of the invention include sequences that are degenerate as a result of the genetic code. All degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of CD1d polypeptide which is encoded by the nucleotide sequence is functionally unchanged in terms of the ability to bind glycolipids.

Minor modifications of the CD1d primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the CD1d polypeptide described herein. Such proteins include those as defined by having essentially the amino acid sequence of SEQ ID NO:2. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of CD1d still exists. Further, deletions of one or more amino acids can also result in modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for the CD1d biological activity of binding glycolipid.

The CD1d polypeptide of the invention encoded by the nucleotide sequence of the invention includes the disclosed sequence (SEQ ID NO:2) and conservative variations thereof, including the sequences of Accession Nos.: NM_007640, NM_007639, X13170, AK002582, M63697, L38820 or P15813. The term "conservative variation" as used herein denotes the replacement of an amino acid

residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, or methionine for another, or the substitution of one solar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

The DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on cDNA using primers capable of annealing to the DNA sequence of interest, and 3) site-directed mutagenesis.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertaining to cells expressing CD1d polypeptides include vectors, preferably expression vectors, containing a CD1d nucleic acid or a portion thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However,

the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors comprise a nucleic acid encoding CD1d or IL2 in a form suitable for expression of the nucleic acid molecule in a host cell. For example, the recombinant expression vectors can include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described in, for example, Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., CD1d proteins, other variant forms of CD1d proteins, fusion proteins, and the like, and IL2 proteins, other variant forms of IL2 proteins, fusion proteins, and the like).

The recombinant expression vectors can be designed for expression of CD1d or IL2 polypeptides in prokaryotic or eukaryotic cells. For example, CD1d polypeptides can be expressed in bacterial cells such as E. Coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the

recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in E. Coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. And Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

One strategy to maximize recombinant protein expression in E. Coli is to express the polypeptide in a bacterial host with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in E. Coli (Wada et al., (1992) Nucleic Acids Res. 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The CD1d expression vector can be a yeast expression vector. Examples of vectors for expression in yeast S. Cerivisae include pYepSec1 (Baldari, et al., (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943),

pJRY88 (Schultz et al., (1987) Gene 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.)

Alternatively, CD1d polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39

A nucleic acid encoding CD1d can be expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989

The recombinant mammalian expression vector is capable of directing expression of the nucleic acid molecule preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid molecule). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733) and immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477), pancreas-specific promoters (Edlund et al. (1 985) Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also

encompassed, for example the murine hox promoters (Kessel and Gruss (1990) Science 249:374-379) and the alpha-fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537-546).

Recombinant expression vectors can be used to produce cells expressing CD1d or IL2 comprising a DNA molecule of CD1d or IL2, respectively, cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to CD1d mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. Et al., Antisense RNA as a molecular tool for genetic analysis, Reviews--Trends in Genetics, Vol. 1(1) 1986

Another aspect pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a CD1d polypeptide can be expressed in bacterial cells such as E. Coli, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an CD1d protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die.

A host cell used in the present invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a CD1d polypeptide. Accordingly the method comprises culturing the host cell (into which a recombinant expression vector encoding an CD1d has been introduced) in a suitable medium such that an CD1d is produced. In another aspect, the method further comprises isolating a CD1d polypeptide from the medium or the host cell.

Similarly, IL2 expression vectors, and cDNA may be constructed, transfected, propagated in host cell lines. IL2 vectors are known in the art and one of skill in the art would be able to readily identify the proper IL2 cDNAs for use in the methods disclosed herein having the benefit of this disclosure. Suitable examples of IL2 sequences and expression vectors may be found, for example, in US Patent

Application Publication 20030207832 and 20030129169, which are incorporated herein by reference in their entirety. IL2 protein may be made by standard techniques known to those skilled in the art. The purified IL2 may be used in the methods disclosed herein. As used herein, "purified" or "substantially pure" means that the predominant species present (i.e., on a molar basis) is more abundant than any other individual species in the composition, and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

IV. Methods of Treatment

As discussed above, the invention provides therapeutic methods and compositions for the prevention and treatment of autoimmune disease and related conditions such as AIDS and organ/tissue rejection. In particular, the invention provides methods and compositions for the prevention and treatment of autoimmune and related diseases in humans as well as other animals through the administration of one or more beta Galactosyl Ceramide compounds. The invention also provides methods of reducing tumor sizes and metastases.

In one embodiment, the present invention contemplates a method of treatment, comprising: a) providing: i) a mammalian patient particularly human who is either at risk for autoimmune disease or who has symptoms of autoimmune disease, ii) one or more beta Galactosyl Ceramide compounds, and; b) administering the one or more beta Galactosyl Ceramide compounds to the patient.

The term "symptoms of autoimmune disease" is herein defined as any abnormal symptoms than can be attributed to the generation of autoreactive B and/or T cells. For example, autoantibodies are a common symptom associated with autoimmune disease.

The term "at risk for autoimmune disease" is herein defined as individuals with familial incidence of autoimmunity. For example, many autoimmune diseases are associated with genetic factors such as certain HLA specificities.

The term "autoimmune and related diseases" refers to those disease states and conditions wherein the immune response of the patient is directed against the patient's own constituents, resulting in an undesirable and often terribly debilitating condition. As used herein, "autoimmune disease" is intended to further include autoimmune conditions, syndromes and the like. An "autoantigen" is a patient's self-produced constituent, which is perceived to be foreign or undesirable, thus triggering an autoimmune response in the patient, which may in turn lead to a chain of events, including the synthesis of other autoantigens or autoantibodies. An "autoantibody" is an antibody produced by an autoimmune patient to one or more of his own constituents which are perceived to be antigenic. For example, in AIDS disease the patient eventually produces autoantibodies to CD4 cells, in SLE autoantibodies are produced to DNA, while in many other types of autoimmune diseases autoantibodies are produced to target cells.

The term "metastasis" refers to tumors that have spread from cancers elsewhere in the body. Reducing tumor size as used herein refers to reducing the cell numbers of the tumor, the weight, the circumference, or the mass of the tumor. Reducing metastasis refers to reducing the sloughing off of tumor cells from tumors and reducing the ability of tumor cell to adhere to other organs.

The present invention is also not limited by the degree of benefit achieved by the administration of beta Galactosyl Ceramide compounds. For example, the present invention is not limited to circumstances where all symptoms are eliminated. In one embodiment, administering a beta Galactosyl Ceramide compound reduces the number or severity of symptoms of an autoimmune disease (e.g., the amount of autoantibody is reduced and/or the amount of pain is reduced). In another embodiment, administering of a beta Galactosyl Ceramide compound may delay the onset of symptoms.

As mentioned above, the indications for which the administration of beta Galactosyl Ceramide compounds can be used include in particular autoimmune conditions; HIV infection/AIDS conditions; and conditions associated with or causing transplant rejection, reducing tumor sizes and reducing metastases, e.g., treatment (including amelioration, reduction, elimination or cure of etiology or symptoms) or prevention (including substantial or complete restriction, prophylaxis or avoidance) of the following:

A) Autoimmune disease and inflammatory conditions, in particular inflammatory conditions with an etiology including an immunological or autoimmune component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and other rheumatic diseases. Specific autoimmune diseases for which the synergistic combination of the invention may be employed include, autoimmune hematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, (autoimmune) inflammatory bowel disease (including e.g. Ulcerative colitis and Crohn's disease), endocrine ophthalmopathy, Graves disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g. Including idiopathic nephrotic syndrome or minimal change nephropathy) and juvenile dermatomyositis. Autoimmune and inflammatory conditions of the skin are also considered to be amenable to treatment and prevention using the synergistic combination of the invention, e.g., psoriasis, contact dermatitis, atopic dermatitis, alopecia areata, erythema multiforma, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, lupus erythematosus, pemphigus, epidermolysis bullosa acquisita, and other inflammatory or allergic conditions of the skin, as are inflammatory conditions of the lungs and airways including asthma, allergies, and pneumoconiosis;

B) HIV infection and all related conditions from initial infection to the full complement of AIDS disease and all other AIDS-related disorders (e.g., ARC);

C) Acute organ or tissue transplant rejection, e.g., treatment of recipients of, e.g., heart, lung, combined heart-lung, liver, kidney, pancreatic, skin, bowel, or corneal transplants, especially prevention and/or treatment of T-cell mediated rejection, as well as graft-versus-host disease, such as following bone marrow transplantation; and

D) Chronic rejection of a transplanted organ, in particular, prevention of graft vessel disease, e.g., characterized by stenosis of the arteries of the graft as a result of intima thickening due to smooth muscle cell proliferation and associated effects.

For the purposes of the present invention, "cytokines" are defined as intercellular mediators secreted by the lymphocytes and/or macrophages. For example, cytokines play a role in the generation of an immune response, such as in an immune response to an infection or infectious organism. Cytokines including, for example, interferons (IFN alpha and IFN gamma) and TNFs induce other cytokines which participate in the development of different autoimmune conditions and diseases. In the development of anti-cytokine therapy in accordance with the present invention, considerable emphasis has been placed on these three cytokines, because it appears that by neutralizing these key cytokines (IFN alpha , IFN gamma and TNF), it is possible to decrease, halt or prevent the synthesis of the cytokines induced by them. However, in certain autoimmune conditions or diseases, including IDDM and SLE, the induction of another cytokine (interleukins, specifically IL-6) is so great and exerts such a strong pathological influence, that it is desirable to remove the cellular source of cytokines which in the context of the present invention, is NKT cells.

Typical subjects for treatment in accordance with the individuals include mammals, such as primates, preferably humans. Cells treated in accordance with the invention also preferably are mammalian, particularly primate, especially human. As discussed above, a subject or cells are suitably identified as in needed of treatment, and the identified cells or subject are then selected for treatment and administered one or more of beta Galactosyl Ceramide compounds.

The treatment methods and compositions of the invention also will be useful for treatment of mammals other than humans, including for veterinary applications such as to treat horses and livestock e.g. cattle, sheep, cows, goats, swine and the like, and pets such as dogs and cats.

For diagnostic or research applications, a wide variety of mammals will be suitable subjects including rodents (e.g. mice, rats, hamsters), rabbits, primates and swine such as inbred pigs and the like. Additionally, for in vitro applications, such as in vitro diagnostic and research applications, body fluids (e.g., blood, plasma, serum, cellular interstitial fluid, saliva, feces and urine) and cell and tissue samples of the above subjects will be suitable for use.

One or more beta Galactosyl Ceramide compounds may be administered as a "cocktail" formulation with other therapeutics, e.g., coordinated administration of one or more compounds of the invention together with one or more other active therapeutics, such as one or more other agents used to treat immune disorders, and/or one or more agents used to treat HIV infections. For instance, a beta Galactosyl Ceramide compound may be administered in coordination with beta interferon or other immune therapy agent, or agents used for treatment of retroviral infections such as a protease inhibitor (e.g., saquinavir, ritonavir, indinavir or AG1343 (Viracept)), AZT, ddI, ddC, d4T, 3TC, FTC, DAPD, acyclovir, and the like.

V. Anti-Proliferative Therapies

One or more beta Galactosyl Ceramide compounds may be useful for treating tumors, for example, one or more beta Galactosyl Ceramide compounds may be administered to a subject with a tumor to reduce the size of the tumor and/or reduce the metastasis of the tumor. One or more beta Galactosyl Ceramide compounds may also be administered with one or more cytokines to reduce the size of the tumor and/or reduce the metastasis of the tumor. Administration protocols for administering one or more beta Galactosyl Ceramide compounds for the reduction or tumor size and/or reduction of metastasis is similar to that for preventing autoimmune diseases.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-.alpha. and .gamma.; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-.beta.; platelet-growth factor; transforming growth factors (TGFs) such as TGF-.alpha. and TGF-.beta.; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-.alpha., -.beta., and -.gamma.; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1a, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-.alpha. or TNF-.beta.; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines. Preferred is IL-2.

As described above, tumor types that may be reduced or metastasis reduced or eliminated are, for example, gastrointestinal malignancies, including colorectal, appendiceal, anal, or small bowel cancers; urogenital malignancies, including prostate, bladder, testicular, or penile cancers; gynecologic malignancies, including cervical, endometrial, ovarian, vaginal, or vulvar cancers; osteogenic and other sarcomatous malignancies in which pelvic structures are involved.

One or more beta Galactosyl Ceramide compounds may be administered prior to, during, and/or after other treatment therapies, for example surgery or radiation therapy. One or more beta Galactosyl Ceramide compounds may be administered, for example, twice a day, three times a day, or four times a day. The one or more beta

Galactosyl Ceramide compounds may be administered, for example, in tablet form, powered form, liquid for or in capsules.

Cytokines, may be administered as cDNA or protein and may be administered with one or more beta Galactosyl Ceramide compounds to treat tumors to reduce their size or to reduce metastases. One or more beta Galactosyl Ceramide compounds administered with, for example, IL2 may also be used in conjunction with other anti-proliferative compounds to treat tumor metastasis,or other compounds, including for example, chemotherapeutic agents, anti-inflammatory agents, anti-pyretic agents radiosensitizing agents, radioprotective agents, urologic agents, anti-emetic agents, and/or anti-diarrheal agents. For example, cisplatin, carboplatin, docetaxel, paclitaxel, flurouracil, capecitabine, gemcitabine, irinotecan, topotecan, etoposide, mitomycin, gefitinib, vincristine, vinblastine, doxorubicin, cyclophosphamide, celecoxib, rofecoxib, valdecoxib, ibuprofen, naproxen, ketoprofen, dexamethasone, prednisone, prednisolone, hydrocortisone, acetaminophen, misonidazole, amifostine, tamsulosin, phenazopyridine, ondansetron, granisetron, alosetron, palonosetron, promethazine, prochlorperazine, trimethobenzamide, aprepitant, diphenoxylate with atropine, and/or loperamide.

Tumor cell types that may respond and benefit from the above described methods include, for example, liver and kidney tumors, hemangiosarcoma, acoustic neuroma, adenoma, astrocytoma, brain stem glioma, chordoma, choroid plexus, craniopharyngioma, ependymoma, ganglioglioma, gangliglioneurocytoma, glioblastoma multiforme (gbm), glioma, lymphoma, medulloblastoma, meningioma - meningiomas & benign brain tumors, neurofibramatosis, oligodendroglioma, optic nerve glioma, pituitary tumors - pituitary tumors, pineal tumors, pineoblastoma, vascular brain tumors, neuroectodermal, adrenocortical carcinoma, adrenocortical carcinoma, childhood, aids-related lymphoma, anal cancer, ductal carcinoma in situ, lobular carcinoma in situ, stage i, ii, and iiia breast cancer, stage iiib, iv, recurrent, and metastatic breast cancer, colon cancer, colorectal cancer, childhood, bladder cancer. Hemangiosarcoma (HSA) is a tumor of blood vessels. Since blood vessels are essential in tumor metastasis, HSA spreads early and aggressively, even when metastatic lesions are not apparent at initial presentation. The most common sites for

HSA are the spleen, liver, right atrium (heart), and skin. The tumor spreads to the lung, most frequently, but can also metastasize to the brain or any other organ.

The administration of one or more beta Galactosyl Ceramide compounds may be to a subject at risk for developing a tumor to prevent the tumor, or alternately the one or more beta Galactosyl Ceramide compounds may be administered to a subject that have been diagnosed with a tumor.

The cytokines may be administered with the one or more beta Galactosyl Ceramide compounds, or they may be administered at a different time than the one or more beta Galactosyl Ceramide compounds. For example, the one or more beta Galactosyl Ceramide compounds may be administered in the morning and the cytokine may be administered in the evening, or the one or more beta Galactosyl Ceramide compounds and the cytokines may be administer at the same time. Either or both may be administered once per day or more than one time per day.

Animal species useful for determining the efficacy of the compounds of the invention include, immunodeficient mice and, in particular, nude and scid mice. The observation that the nude mouse with hypo/aplasia could successfully act as a host for human tumor xenografts has lead to its widespread use for this purpose. The autosomal recessive nu gene has been introduced into a very large number of distinct congenic strains of nude mice, including, for example, ASW, A/He, AKR, BALB/c, BIO.LP, C17, C3H, C57BL, C57, CBA, DBA, DDD, I/st, NC, NFR, NFS, NFS/N, NZB, NZC, NZW, P, RIII and SJL. In addition, a wide variety of other animals with inherited immunological defects other than the nude mouse have been bred and used as recipients of tumor xenografts. For further details see, e.g., The Nude Mouse in Oncology Research, E. Boven and B. Winograd, eds., CRC Press, Inc., 1991.

The cells introduced into such animals can be derived from known tumor/cancer cell lines, such as, any of the above-listed tumor cell lines, and, for example, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the neu protooncogene); ras-transfected NIH-3T3 cells; Caco-2 (ATCC HTB-37); a moderately well differentiated grade II human colon adenocarcinoma cell line, HT-29 (ATCC HTB-3 8), or from tumors and cancers. Samples of tumor or cancer cells can

be obtained from patients undergoing surgery, using standard conditions, involving freezing and storing in liquid nitrogen (Karmali et al., Br. J. Cancer. 48:689-696 [1983]).

Tumor cells can be introduced into animals, such as nude mice, by a variety of procedures. The subcutaneous (s.c.) space in mice is very suitable for tumor implantation. Tumors can be transplanted s.c. as solid blocks, as needle biopsies by use of a trochar, or as cell suspensions. For solid block or trochar implantation, tumor tissue fragments of suitable size are introduced into the s.c. space. Cell suspensions are freshly prepared from primary tumors or stable tumor cell lines, and injected subcutaneously. Tumor cells can also be injected as subdermal implants. In this location, the inoculum is deposited between the lower part of the dermal connective tissue and the s.c. tissue. Boven and Winograd (1991), supra. Animal models of breast cancer can be generated, for example, by implanting rat neuroblastoma cells (from which the neu oncogen was initially isolated), or neutransformed NIH-3T3 cells into nude mice, essentially as described by Drebin et al., Proc. Natl. Acad. Sci. USA 83:9129-9133 (1986).

Similarly, animal models of colon cancer can be generated by passaging colon cancer cells in animals, e.g., nude mice, leading to the appearance of tumors in these animals. An orthotopic transplant model of human colon cancer in nude mice has been described, for example, by Wang et al., Cancer Research 54:4726-4728 (1994) and Too et al., Cancer Research, 55:681-684 (1995). This model is based on the so-called "METAMOUSE" sold by AntiCancer, Inc., (San Diego, Calif.). Tumors that arise in animals can be removed and cultured in vitro. Cells from the in vitro cultures can then be passaged to animals. Such tumors can serve as targets for further testing or drug screening. Alternatively, the tumors resulting from the passage can be isolated and RNA from pre-passage cells and cells isolated after one or more rounds of passage analyzed for differential expression of genes of interest. Such passaging techniques can be performed with any known tumor or cancer cell lines.

Also useful for screening the compounds of the invention are the Meth A, CMS4, CMS5, CMS21, and WEHI-164 are chemically induced fibrosarcomas of BALB/c female mice (DeLeo et al., J. Exp. Med., 146:720 [1977]), which provide a

highly controllable model system for studying the anti-tumor activities of various agents (Palladino et al., J. Immunol., 138:4023-4032 [1987]). Briefly, tumor cells are propagated in vitro in cell culture. Prior to injection into the animals, the cell lines are washed and suspended in buffer, at a cell density of about 10.times.106 to 10.times.10' cells/ml. The animals are then infected subcutaneously with 10 to 100 kit of the cell suspension, allowing one to three weeks for a tumor to appear.

In addition, the Lewis lung (3LL) carcinoma of mice is also useful, which is one of the most thoroughly studied experimental tumors, can be used as an investigational tumor model. Efficacy in this tumor model has been correlated with beneficial effects in the treatment of human patients diagnosed with small cell carcinoma of the lung (SCCL). This tumor can be introduced in normal mice upon injection of tumor fragments from an affected mouse or of cells maintained in culture (Zupi et al., Br. J. Cancer, 41, suppl. 4:309 [1980]), and evidence indicates that tumors can be started from injection of even a single cell and that a very high proportion of infected tumor cells survive. For further information about this tumor model see, Zacharski, Haemostasis. 16:300-320 [1986]). Also useful are the cell types used in the examples, which follow.

VI. Pharmaceutical Compositions

The beta Galactosyl Ceramide compounds (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule or the protein modulator and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. As discussed above, supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal,

subcutaneous, oral, inhalation, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. PH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL.TM. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The composition preferably is sterile and should be fluid to the extent that easy syringability exists. The compositions suitably should be stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., beta Galactosyl Ceramide compound, an CD1d nucleic acid molecule, an CD1d protein, or anti-CD1d antibody) in a therapeutically effective or beneficial amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. Suitable oral compositions may be e.g., enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier

to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially e.g. from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

Data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within-this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The pharmaceutical compositions can be included in a container, pack, kit or dispenser together with instructions (e.g. written) for administration, particularly such instructions for use of the active agent to treat against a disorder or disease as disclosed herein, including an autoimmune disease or disorder, treatment in connection with an organ or tissue transplant, as well as other diseases or disorders with an autoimmune component such as AIDS. The container, pack, kit or dispenser may also contain CD1d polypeptide, a nucleic acid sequence encoding a CD1d peptide, or a CD1d-expressing cell.

The following non-limiting examples are illustrative of the invention. All documents mentioned herein are incorporated herein by reference in their entirety.

EXAMPLES

Materials and Methods used in the Examples:

NK cell isolation. Liver or spleen NK cells were isolated from C57BL/6 (B6) mice and grown for 7-10 days in 1000 IU/ml recombinant IL-2 (Chiron Corp.). Liver mononuclear cells were used either fresh or after IL-2 expansion and were 35-70% $CD3^-$, $NK1.1^+$.

Flow Reagents and Antibodies used. NK1.1-PE (or APC), DX-5-PE and CD3_-PcP (Becton Dickinson-Pharmingen, San Jose, CA) as well as CD69-FITC were used for flow cytometric analysis. IFN-γ cytoplasmic detection was performed using kits purchased from Becton Dickinson/Pharmingen. Annexin V was used to evaluate early apoptosis events [Becton Dickinson/Pharmingen].

Flow cytometry analysis (FCA) and Sorting. Cells were stained and analyzed on a LSR flow cytometer (Becton Dickinson, San Jose, CA). Cells were directly stained using FITC-, phycoerythrin (PE-), Per-CP- and APC-labeled primary abs. Sorting experiments were performed on a MoFlo cell sorter (Dako/Cytomation, Ft. Collins, CO) to isolate NK, and NKT cells using NK1.1-PE and CD3biotin-SA.Tricolor.

Cytokine measurement. Cytokines were measured using IFN-γ, and chemokine ELISA kits (R&D Systems, Minneapolis, MN). Cell stimulations were performed at cell concentrations of $1\text{-}5x10^6$ cells/ml. In all assays, the standard deviation was less than 5 pg/ml. In some experiments, serum levels were evaluated. In experiments using A20 and A20/CD1d (provided by M. Kronenberg, LaJolla Inst., San Diego, CA), these B cell lines were pretreated with agents for 30 min at 37 C, washed and mixed with sorted populations of NK or NKT cells (>98% pure) and supernatant were collected after 24 hrs.

Ribonuclease protection assay. The multiprobe RNAse Protection Assay was performed utilizing the mck-1 or mck-5 probe set (Pharmingen, San Diego, CA). Total cellular RNA was extracted utilizing Trisol (Life Technologies, Gaithersburg, MD) and 1-5 μg of total mRNA was hybridized with a ^{33}P UTP labeled RNA probe (1 x 10^6 cpm/sample) prepared according to the manufacturers directions (Pharmingen, La Jolla, CA) using the Pharmingen RiboQuant In Vitro Transcription kit. Following hybridization, the samples were treated with RNAse A and T1 according to the procedure provided by Pharmingen. The RNAse was inactivated and precipitated utilizing a master cocktail containing 200 μl Ambion (Austin, TX) RNAse inactivation reagent, 50 μl ethanol, 5 μg yeast tRNA and 1 μl Ambion GycoBlue co-precipitate per RNA sample. The samples were mixed well, incubated at -70EC for 15 min and centrifuged at 14,000 rpm for 15 min at room temperature. The pellets were suspended in 3 μl of Pharmingen sample buffer and subjected to polyacrylamide gel electrophoresis as recommended by the manufacturer (Pharmingen).

Cytotoxicity Assay. NK cytotoxicity was evaluated using Yac-1 prototype NK target. Cytotoxicity was measured in a standard 4-hr ^{51}Cr-release assay.

Mice used in this study. Mice were obtained from the Animal Production Area, NCI-FCRDC and used between 6-12 weeks of age.

Ceramide Reagents: Various reagents were purchased from AvantiLipids, Alabaster, AL 35007. D-Glucosyl-ß1-1' Ceramide (C8), D-Galactosyl-ß1-1' Ceramide (C8), D-Glucosyl-β1-1' Ceramide (C12), and D-Galactosyl-ß1-1' Ceramide (C12). In addition, alpha-galactosyl-ceramide (KRN7000) was graciously provided by Kirin, Japan. The ceramide reagents were first dissolved in DMSO, then diluted in PBS containing 0.5% tween 20. Control diluent was used for untreated mice.

Bone Marrow Transplantation. Marrow transplantation was evaluated using a new label tracking (L. Öberg, Abstract: Soc. Natural Immunity Meeting, San Juan, PR, 2002) procedure that utilizes CFSE (Molecular Probes) labeled bone marrow cells that are evaluated after 1-4 days. Briefly, 10x10^6 CFSE labeled bone marrow cells that are injected into irradiated recipient mice (C57BL/6 - 900R; Balb/c - 800R). The spleens are evaluated for the total number of labeled cells in the autologous

versus the allogeneic transfer group with or without treatment. Mice are evaluated individually and the mean and standard deviation for each group is calculated.

Tetrameric GalCer/CD1d complexes. Tetramers of wt CD1d molecules were produced as described by Matsuda et al. (19), using sequences of Accession Nos.: NM_007640, NM_007639, X13170, AK002582, M63697, L38820 or P15813. CD1d molecules were biotinylated and prepared by the NIH tetramer facility. Biotinylated CD1d molecules were then incubated overnight at room temperature with a 3-fold molar excess of α or βGalCer (solubilized in 0.5% Tween 20, 0.9% sodium chloride, hereafter called vehicle) or with an equal amount of vehicle alone. CD1d monomers were then tetramerized using a 1:4 molar ratio of APC-conjugated streptavidin (BD PharMingen, San Diego, CA).

Tetramer staining. All staining and washes were performed in a buffer consisting of 2% FCS, 0.075% sodium bicarbonate, and 0.1% sodium azide in RPMI medium (Sigma-Aldrich, St. Louis, MO), as previous described (19). Cells were stained for 3h at room temperature, then stained with subsets antibodies, washed two times and fixed in 1% paraformaldehyde (Sigma- Aldrich) in PBS. The intensity of fluorescence on hybridoma cells was determined by flow cytometry analysis using a FACSort flow cytometer (BD Biosciences, Mountain View, CA).

Example 1: Evaluation of Alpha Galactosyl Ceramide *In Vivo.*

To evaluate the in vivo effects of administration of αGalCer on immune functions, we administered several doses this agent (10, 1 and 0.1 ug/mouse) and evaluated an variety of parameters that are depicted in Figure 1. When we examined the effect on NK cells, we could see a rapid and dramatic activation of NK cells as enumerated by increase in NK lysis (panel A), increase in the size of NK cells (panel B), increase of the activation antigen, CD69 (panel C), increase in the total number of NK cells (panel D), increase in the percent of NK cells (panel F), and the expected decrease in NKT cells numbers (panel E) and percent (panel G). In addition at the highest doses of αGalCer we observed a general increase in liver cellularity (2 - 10 fold) and a rapid and potent production of serum IFNγ that was observed up to 48 hrs. Of interest was the wide range of doses of αGalCer that resulted in potent and sustained activation of NK cells (beyond 7 days in lytic function - panel A) and

increase in NK cells numbers and frequency (panels D & F). These results substantiate and extend previous observations that the administration of αGalCer in vivo results in a rapid loss of a major NKT subset from the liver and results in a bystander activation of NK cells. These findings demonstrate that this NK activation is quite rapid, long lasting and multiparametered.

Example 2: Evaluation of Alpha Galactosyl Ceramide in SCID Mice.

To demonstrate that the NKT cells were responsible for the initiation of the bystander NK activation, experiments were performed in SCID mice (lacking NKT cells). C57BL/6 or C57BL/6 SCID mice were analyzed for the ability of 1 μg/ml αGalCer to increase NK cell size (left panel) or NK activity (right panel). Cytotoxicity (and SD) are shown in line chart with lytic unit values indicated in the legend. As shown in Figure 2, the administration of αGalCer in SCID mice failed to increase NK cell size or cytolytic activity whereas expected increases, in both cell size and NK lytic potential were seen in control C57BL/6 mice. Other parameters shown in Figure 1 were also not observed in SCID mice. These results strongly implicate activation of NKT cells as a prerequisite for subsequent NK cell activation after administration of αGalCer.

Example 3: Evaluation of Alpha Galactosyl Ceramide Activation of NK Cells.

To determine some mechanistic aspect of the αGalCer activation of NK cells, we performed these treatments in a variety of knockout mice that lacked potential important molecules in immune regulation (Fas, FasL, TNFα, MIP1α, IFNγ, IL12 and CD40L). Results of representative experiments are shown in Table 1. The evaluation of NKT depletion in these knockout mice indicated that strong depletion was observed at 24 hrs in all strains. Some knockout mice, like FasL and CD40L, had low percentages of NKT cells, but loss of >50% of these cells was observed by αGalCer treatment. When NK bystander activation was evaluated at 24 hrs, activation of NK lysis and size (as well as CD69) was observed in all knockout mice (PFP mice lacked NK lysis). However, potent increases in NK frequency and numbers were not observed in FasL knockout mice suggesting a role for the signaling pathway in the bystander effect. It should be noted that 24 hrs is an early time point for evaluation of

cellular increases as these increases are generally maximal at 3-5 days after αGalCer administration. Of particular interest was that IFNγ knockout mice demonstrate NK bystander activation, indicating that IFNγ was not required for at least some of the bystander effects, and suggesting that the activation of NK cells may not be directly linked to the burst of IFN-γ that occurs coincident with NKT cell depletion.

Example 4: Dissociation between NKT depletion and NK augmentation by βGalCer (C12).

The results shown above re-emphasize the coordinate induction of two quite different αGalCer-induced regulatory events in key innate immune parameters, the reduction of NKT cells and the activation of NK cells. This data set demonstrates the potency of αGalCer for stimulating innate immune functions, but also illustrates the difficulties in using αGalCer to discriminate the roles of these two cell types in various biological effects in vivo.

Studies were performed to evaluate ceramides to deplete NKT cells without causing downstream activation events (e.g. NK activation and IFN-γ production). The data presented in Table II shows that βGalCer (C12) potently depletes detectable NKT cells, while other ceramide compounds including βGalCer (C8), β-GlucCer (C12), and β-GlucCer (C8) demonstrated no biological activity when compared to the diluent control. As shown in both Table II and Figure 3, administration of βGalCer (C12) resulted in a similar reduction in detectable NKT cells as seen for αGalCer (panel D), but without the broad bystander NK activation obtained after administration of αGalCer, as illustrated by a lack of increased percentage, lysis and CD69 expression (panels A-C, respectively). High doses of βGalCer (C12) were utilized to ensure that maximal depletion and/or potential activation would be detected. Overall, it is clear that βGalCer (C12) can effectively reduce the number of detectable NKT cells, and this effect can be mediated in the absence of the NKT dependent bystander NK activation that occurs after administration of αGalCer.

Example 5: βGalCer (C12) reduces the number of detectable NKT cells in the absence of TH_1 and TH_2 cytokine induction.

We have demonstrated (Table I, Figure 2) that αGalCer activation and subsequent reduction in detectable of NKT cells results in strong IFN-γ production. We evaluated the abilities of αGalCer vs βGalCer (C12) to induce cytokine production in vivo. Cytokine gene expression for IL-4, IL-13 or IFN-γ was evaluated in both liver and spleen 1, 2 or 4 h after administration of αGalCer and βGalCer (C12). As shown in Figure 4, only αGalCer induced IL-4, IL-13, or IFN-γ. Interestingly, the ability of αGalCer to increase gene expression at 2 h and/or 4 h was demonstrated in both the liver that contains large numbers of NKT cells (15-30% of all lymphocytes), as well as in the spleen that generally contains only 1-3% NKT cells. Thus, although both αGalCer and βGalCer (C12) reduce NKT cell detection, only αGalCer induces cytokine gene expression in spleen and liver. These results were extended by further studies where the ability of αGalCer and βGalCer (C12) to induce cytokine proteins in the serum were studied. The results shown in Figure 5 demonstrate that αGalCer potently increases serum levels of IFN-γ and GM-CSF (panel A) and IL-4 and TNFα (panel B) at 1, 2 or 4 hours after treatment, whereas βGalCer (C12) did not induce appreciable amounts of these cytokines. The rapid induction of cytokine genes and proteins by αGalCer suggests that these effects must be closely linked to the binding and activation of αGalCer to the TcR of NKT cells. However, the failure of βGalCer (C12) to induce cytokine gene expression while effectively depleting NKT cells contrasts sharply with the effects of αGalCer. This apparent dichotomy of activities for these two ceramides suggests on one hand a similar pathway for stimulating NKT cells, but on the other hand a downstream divergence in signaling for cytokine gene expression. In order to investigate these questions, studies were performed to compare the abilities of αGalCer vs βGalCer (C12) to induce Annexin V expression on NKT cells as an early measure of apoptosis induction. The results shown in Figure 5, panel C demonstrate that both αGalCer and βGalCer (C12) rapidly reduce the number of NKT cells (by about 50% within 1.5 h) also induce a rapid increase in Annexin V expression on NKT, but not NK cells (panel D). The simultaneous induction of both Annexin V expression and loss of NKT detection for both αGalCer and βGalCer (C12) suggests a similar molecular

mechanism for these effects by both agents. Examination of NK cell numbers over this time period demonstrated no increase in Annexin V expression for either αGalCer or βGalCer (C12). Thus, βGalCer (C12) can be used to dissociate NK activating events from the process of NKT apoptosis and reduction, suggesting that the quality of the signals induced by βGalCer (C12) and αGalCer binding to the TcR differ.

Example 6: Cd1d development binding ofαGalCer and βGalCer (C12) to NKT cells.

Since the αGalCer resulted in such a rapid and pronounced increase of NK cell activation and cytokine induction whereas βGalCer failed to induce this potent stimulation, we evaluated the relative efficiency of binding of both ceramides to NKT cells. The results of a typical binding study are shown in Figure 6. CD1d tetramers were either loaded with αGalCer or βGalCer (C12) and their binding to NKT-enriched liver lymphocytes was studied. Figure 9a reveals the strong binding of αGalCer-loaded CD1d to NKT cells (bold line), and this was not observed with either NK cells or NK1.1$^-$ T cells. Figure 9b shows binding with βGalCer (C12)-loaded tetramers were a similar percentage of NKT cells bound the CD1d, albeit at lower intensity, while both NK and NK1.1$^-$ T cells failed to demonstrate this binding. When NKT subsets were evaluated, both CD4 and CD8 subsets bound αGalCer with a strong intensity (Figure 9c and d), while βGalCer (C12) binding to CD4$^+$ NKT cells was significantly stronger than it was to CD8$^+$ cells (Figure 9d). Binding of unloaded tetramers was used as a control in Figure 9c and d. Similar experiments were performed with flow sorted NKT cells that had been sorted and cultured for 3-4 days in IL-2. These results demonstrated a similar intensity difference in binding of αGalCer and βGalCer (C12) to highly purified NKT cells, and a lack of binding of αGalCer to either NK or T cells.

In order to more directly investigate the relative abilities of αGalCer or βGalCer (C12) to directly interact with the TcR of NKT cells, we employed an in vitro assay system in which control A20 cells or A20 cells transfected with CD1d were used to present αGalCer or βGalCer (C12) to isolated NKT vs NK cells (negative control). Cultures containing these enriched subsets of NK or NKT cells and A20 cells were stimulated with αGalCer or βGalCer (C12) for 24h and

supernatants evaluated for cytokine release (Figure 7). IFN-γ and IL-4 production was strongly induced from NKT cells by αGalCer but not βGalCer (C12) only when CD1d transfected A20 cells were used. Only low levels of cytokine production were observed when transfected A20 cells were treated with βGalCer (C12) or when NK cells were stimulated with either αGalCer or βGalCer(C12). These results clearly show that presentation of αGalCer by CD1d is required for cytokine induction, while βGalCer (C12) is either not efficiently presented to NKT cells by CD1d or the quality of the signal is insufficient to induce cytokine production.

Additional studies were also done to evaluate the abilities of αGalCer and βGalCer to activate NKT cells with ceramide presented by peritoneal monocytes. Figure 8 shows a representative experiment where liver lymphocytes (32% NKT) were mixed 1:1 with thioglycolate induced peritoneal exudate cells after loading for 1h at 37°C with varying doses of αGalCer and βGalCer (C12). Similar to the results with the CD1d transfected cell line, αGalCer rapidly induced activation and production of TNFα, IFN-γ, IL-4 and IL-13, while βGalCer induced only low levels of TNFα, and no IFN-γ, IL-4, and IL-13. Both αGalCer and βGalCer (C12) were able to induce IL-5 from liver lymphocytes. Overall, these results show that the biological effects of βGalCer (C12) differ dramatically from those of αGalCer in the context of CD1d and macrophage-mediated presentation to NK cells.

Example 7: βGalCer (C12) depletes NKT cells, but fails to induce rejection of allogeneic bone marrow.

The accumulated results above demonstrate that βGalCer (C12) is efficiently recognized by NKT cells, but does not effectively activate NK cells. This conclusion implies that βGalCer (C12) may not effectively induce cytokines or NK-dependent biological effects in vivo at doses that effectively reduce the number of detectable NKT cells.

Since one of the most sensitive measures of NK activity in vivo is their ability to reject bone marrow, allogeneic transfer of C57BL/6 [H-2^b] marrow into Balb/c [H-2^d] mice was used as a model to confirm that βGalCer (C12) was unable to induce NK-mediated functions in vivo. Balb/c mice are known to be relatively weak bone

marrow rejecters and thus this model can easily detect even a small amount of NK cell activation. The transfer of C57BL/6 bone marrow cells into allogeneic Balb/c mice was more than 50% rejected by 3 days (Figure 9). As expected, this event was shown to be NK-mediated since prior depletion of NK cells using anti-asGM1 serum in the Balb/c recipient mice (which lack NK1.1 expression) reduces the degree of allogeneic graft rejection. The treatment of mice with αGalCer which results in NK activation, further enhanced the rejection of the donor cells to ~90%. In contrast, treatment with βGalCer (C12), which does not efficiently activate NK cells, did not significantly enhance basal rejection. In order to determine whether clear dose-dependent differences could be established for these two agents, additional studies were performed and these showed that αGalCer doses from 0.1 to 0.01 μg/mouse retained NK activating abilities (panel B), while βGalCer (C12) doses from 1 to 10μg/mouse failed to induce any in vitro NK activation. The results shown in panel C confirm that both ceramides had the expected ability to reduce the detection of NKT cells at 24h. Previous studies suggested that even doses of βGalCer (C12) ≥ 10μg resulted in NKT depletion with minimal effects on NK cells. Panel D shows two experiments where several doses of αGalCer and βGalCer (C12) were evaluated for enhancement of marrow rejection. Whereas αGalCer can potently activate marrow rejection at a dose as low as 0.1 μg/mouse, βGalCer (C12) at a dose of 10 μg, which is effective at reducing the number of NKT cells, had little enhancing effect on bone marrow graft rejection. Interestingly, a very high dose of βGalCer (20 μg) which also had no appreciable NK enhancing effects (Figure 3) did have some ability to enhance rejection of bone marrow cells suggesting that at very high doses βGalCer (C12) can either induce enough augmentation of NK activity (see Table II) to mediate some effects in vivo or induce some biological effects through a non-NK-mediated mechanism. However, it is clear that in all studies a dose of 10 μg βGalCer can be used in vivo to effectively deplete NKT cells without appreciable NK-activating effects as assessed by NK number, activation stage, cytolytic activity, or very sensitive NK-dependent functions in vitro.

Overall, these results confirm that βGalCer (C12) does directly bind to NKT cells, but that differences in the intensity and subset affinity of this binding may contribute to its dramatically different in vitro and in vivo biological effects.

Example 8: Mouse Kidney Cancer Model – Co-administration of IL-2.

A mouse kidney cancer model was used to examine the role of NKT cells in liver and lung metastasis originating from the Renca model. In addition to NK cells, NKT cells can complicate interpretation of data involving liver tumors since the NKT cells are highly prominent in the liver. Use of anti-asial-GM1 (which does not remove or alter NKT functions) suggested that NKT are not a major effector in eliminating liver metastases. However, since there are no other antibodies selective for NKT cells, we utilized a compound β-Galactose-Cerimide (C12; βGalCer) that depletes NKT cells in the mouse without activating other cells that may have anti-tumor activity, as does the potential NKT ligand, compound α-Galactose-Cerimide. First we evaluated combination treatments of IL-2 cDNA and NKT depletion on liver metastases (see Figure 10). The administration of α-GalCer resulted in a dramatic decrease in liver tumor ($p<0.01$), presumably due to both the activation of NK cells and removal of NKT cells. When β-GalCer or IL-2 was given to the mice a small reduction (not significant) of tumor was observed. However, when both β-GalCer (to remove NKTs) and cDNA IL2 (to activate NK cells) were co-administered ($p<0.05$), a dramatic reduction in liver metastasis, similar to the reduction seen after administration of α-GalCer was observed. Similar effects combining β-GalCer and cDNA IL2 were seen when the kidney cancer was targeted to the lung by intra-venous injection (not shown). These results demonstrated that the removal of NKT cells with β-GalCer and the co-administration of an activating agent, such as IL-2, result in strong anti-tumor effects.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the disclosure, may make modifications and improvements within the spirit and scope of the invention.

Table I. *Summary of αGalCer effects on liver subsets and function*

		Cell #/ Mouse ($X10^6$)	% NK	%NKT	% T	NK Size (MCF)	NK Lysis	# of NK ($X10^5$)	# of NKT ($X10^5$)	# of T ($X10^6$)
Exp. 1										
NT	B6	3.60	6.5	16.5	34.6	292	38.5	2.3	6.0	1.2
αGal 24	B6	4.40	10.6	1.9	33.2	349	743.0	4.7	0.8	1.5
NT	GLD (FasL)	15.40	2.3	3.0	54.0	325	60.3	3.5	4.5	8.3
αGal 24	GLD (FasL)	9.00	4.4	1.3	62.1	355	329.0	3.9	1.2	5.6
NT	PFP	2.90	7.2	13.2	36.0	289	<1	2.1	3.8	1.0
αGal 24	PFP	3.40	9.6	2.1	44.0	337	<1	3.3	0.7	1.5
Exp.2										
NT	B6	3.00	6.9	28.6	29.6	272	36.0	2.1	8.6	.89
αGal 24	B6	4.10	11.9	1.3	41.2	319	340.0	4.9	0.5	1.7
NT	TNF	3.50	7.7	26.8	29.1	275	82.0	2.7	9.4	1.0
αGal 24	TNF	8.20	4.6	0.9	46.0	325	473.0	3.8	0.7	3.8
NT	MIP	1.90	11.1	13.0	26.0	274	24.1	2.1	2.5	0.5
αGal 24	MIP	3.10	4.6	0.4	32.8	338	662.2	1.4	0.1	1.0
Exp.3										
NT	B6	2.70	5.2	23.1	31.0	276	25.9	1.4	6.2	0.8
αGal 24	B6	2.70	9.5	4.5	40.3	316	714.8	2.6	1.2	1.1
NT	GLD (FasL)	2.30	3.8	7.1	44.5	280	29.1	0.9	1.6	1.0
αGal 24	GLD (FasL)	9.00	4.5	3.2	65.1	320	99.2	4.1	2.9	5.9
Exp.4										
NT	B6	3.45	8.0	23.0	32.1	214	55.0	2.8	7.9	1.1
αGal	B6	2.40	15.8	4.0	37.2	266	830.4	3.8	1.0	0.9
NT	GKO	1.67	5.6	20.0	29.3	207	11.9	0.9	3.3	0.5
αGal	GKO	3.70	14.1	6.5	44.0	268	249.8	5.2	2.4	1.6
NT	IL-12p40	2.80	6.9	13.0	25.6	222	16.8	1.9	5.6	0.7
αGal	IL-12p40	4.00	7.5	3.8	27.9	280	245.0	3.0	1.5	1.1
NT	CD40L	1.80	5.0	7.3	31.5	211	53.7	0.9	1.3	0.6
αGal	CD40L	3.66	6.3	1.7	45.5	272	662.8	2.3	0.6	1.7

* Data from liver at 24 h after administration of 1 μg of αGalCer

Table II. *Comparison of ceramides in their ability to deplete NKT cells and activate NK cells*

Treatment	Dose µg/mouse	% NK	% NKT	NK size	NK CD69	NK Lysis Units
NT		5.2	25.1	237	59	107
αGal	0.1	10.1	**8.9**	**281**	**80**	**1715**
βGal [c8]	10	5.8	24.5	246	57	133
βGal [c8]	1	7.3	23.4	233	44	129
βGal [c12]	10	9.6	**8.2**	245	45	**417**
βGal [c12]	1	11.2	**11.7**	235	41	198
βGlu [c8]	10	4.3	19.2	244	61	48
βGlu [c8]	1	5.3	25.0	242	62	22
βGlu [c12]	10	5.1	21.8	242	58	43
βGlu [c12]	1	4.1	23.3	244	62	44

Bolded values indicate significant increases a decision ($p<0.05$), as compared to the negative control, induced by treatments. Similar results were obtained for all treatments in at least two additional experiments.

We claim:

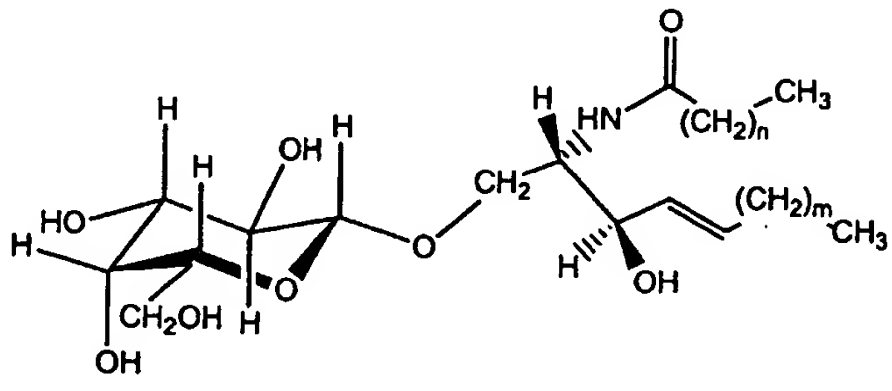
1. A method for treating a subject suffering from or susceptible to an autoimmune disease or disorder, or a disease or disorder having an autoimmune component, comprising administering to the subject an effective amount of one or more beta Galactosyl Ceramide compounds.

2. A method for treating a subject receiving or having received an organ or tissue transplant, comprising administering to the subject an effective amount of one or more beta Galactosyl Ceramide compounds.

3. A method of claim 1 or 2 wherein the administered one or more beta Galactosyl Ceramide compounds provide at least about a five percent decrease in NKT cell amounts relative to the control in a standard *in vitro* NKT assay.

4. A method of any one of claims 1 through 3 wherein administration of the one or more beta Galactosyl Ceramide compounds result in no more about 50 percent cytokine production relative to alpha Galactosyl Ceramide.

5. A method of claims 1 or 2 wherein the beta Galactosyl Ceramide compounds are of the following general formula:



wherein n = 8 to 15 and m = 10 to 20, or a pharmaceutically acceptable salt or solvate thereof.

6. A method of claims 1 or 2 wherein the beta Galactosyl Ceramide compound is D-Galactosyl-β1-1'-N-Dodecanoyl-D-erythroSphingosine; D-Galactosyl-β1-1'-N-Nonanoyl-D-erthroSphingosine; D-Galactosyl-β1-1'-N-Decanoyl-D-erthroSphingosine; D-Galactosyl-β1-1'-N-Ondecanoyl-D-erthroSphingosine; D-Galactosyl-β1-1'-N-Propdecanoyl-D-erthroSphingosine; D-Galactosyl-β1-1'-N-Butdecanoyl-D-erthroSphingosine; or D-Galactosyl-β1-1'-N-Pentdecanoyl-D-erthroSphingosine.

7. A method of any one of claims 1 through 6 wherein the beta Galactosyl Ceramide compound is administered in conjunction with a CD1d polypeptide, a nucleic acid sequence encoding a CD1d peptide, a CD1d-expressing cell, IL-2, IL-2 cDNA, or IL-2 peptide.

8. A method of any one of claims 1 through 7 wherein the subject is suffering from or susceptible to an autoimmune disease or disorder, or disease or disorder having an autoimmune component.

9. A method of any one of claims 1 through 7 wherein the subject is suffering from or susceptible to arthritis, an autoimmune hematological disorder, systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, (autoimmune) inflammatory bowel disease, endocrine ophthalmopathy, Graves disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes, uveitis, keratoconjunctivitis sicca, vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis, juvenile dermatomyositis, psoriasis, contact dermatitis, atopic dermatitis, alopecia areata, erythema multiforma, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, lupus erythematosus, pemphigus, epidermolysis bullosa acquisita, or an inflammatory condition of the lung or airways.

10. A method of any one of claims 1 through 7 wherein the subject is suffering from an HIV infection.

11. A method of any one of claims 1 through 7 wherein the subject is undergoing or has received a tissue or organ transplant.

12. A method of any one of claims 1 through 11 wherein the subject is identified as suffering from or susceptible to the disease or disorder and the identified subject is selected for administration of the beta Galactosyl Ceramide compound.

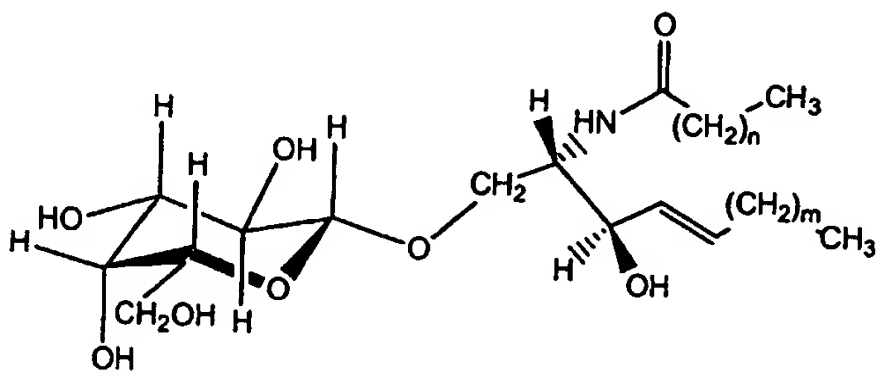
13. A method of any one of claims 1 through 12 wherein the subject is a human.

14. A method for decreasing a population of Natural Killer T cells, comprising administering to mammalian cells or tissue an effective amount of one or more beta Galactosyl Ceramide compounds.

15. The method of claim 14 wherein the one or more beta Galactosyl Ceramide compounds are administered to human cells or tissue.

16. The method of claim 14 wherein the one or more beta Galactosyl Ceramide compounds are administered to a mammal suffering from an autoimmune disease or disorder.

17. The method of any one of claims 14 through 16 wherein the one or more beta Galactosyl Ceramide compounds are of the following general formula:



wherein n = 8 to 15 and m = 10 to 20, or a pharmaceutically acceptable salt or solvate thereof.

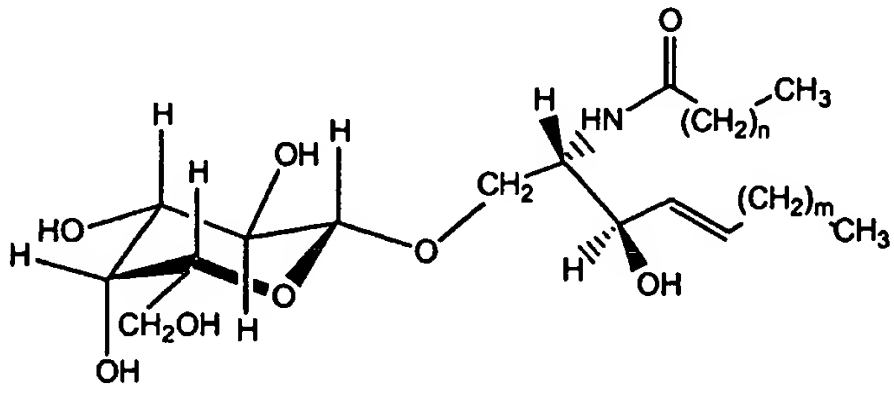
18. A pharmaceutical composition kit comprising i) one or more beta Galactosyl Ceramide compounds; and ii) directions for use of the one or more beta Galactosyl Ceramide compounds to treat autoimmune disease or disorder, or a disease or disorder having an autoimmune component or a subject receiving or having received an organ or tissue transplant.

19. The pharmaceutical composition kit of claim 18 further comprising a CD1d polypeptide, a nucleic acid sequence encoding a CD1d peptide, or a CD1d-expressing cell.

20. The pharmaceutical composition kit of claim 18 further comprising IL-2, IL-2 cDNA, or IL-2 peptide.

21. A method of treating cancer comprising administering to a subject in need thereof a beta Galactosyl Ceramide compound.

22. The method of claim 21, wherein the beta Galactosyl Ceramide compound is of the following general formula:



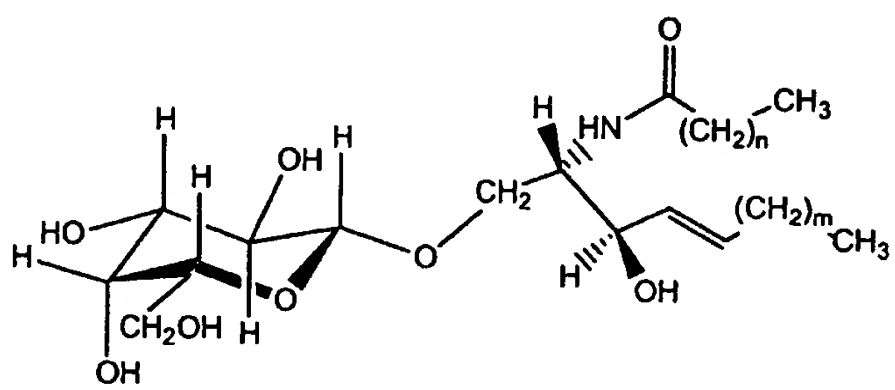
wherein n = 8 to 15 and m = 10 to 20, or a pharmaceutically acceptable salt or solvate thereof.

23. The method of claim 21, further comprising administering a cytokine.

24. The method of claim 23, wherein the cytokine is IL-2.

25. A method to reduce tumor size or metathesis comprising administering to a subject in need thereof a beta Galactosyl Ceramide compound.

26. The method of claim 25, wherein the beta Galactosyl Ceramide compound is of the following general formula:



wherein n = 8 to 15 and m = 10 to 20, or a pharmaceutically acceptable salt or solvate thereof.

27. The method of claim 25, wherein the tumor is a liver or kidney tumor.

28. The method of claim 25, further comprising administering a cytokine.

29. The method of claim 27, wherein the cytokine is IL-2.

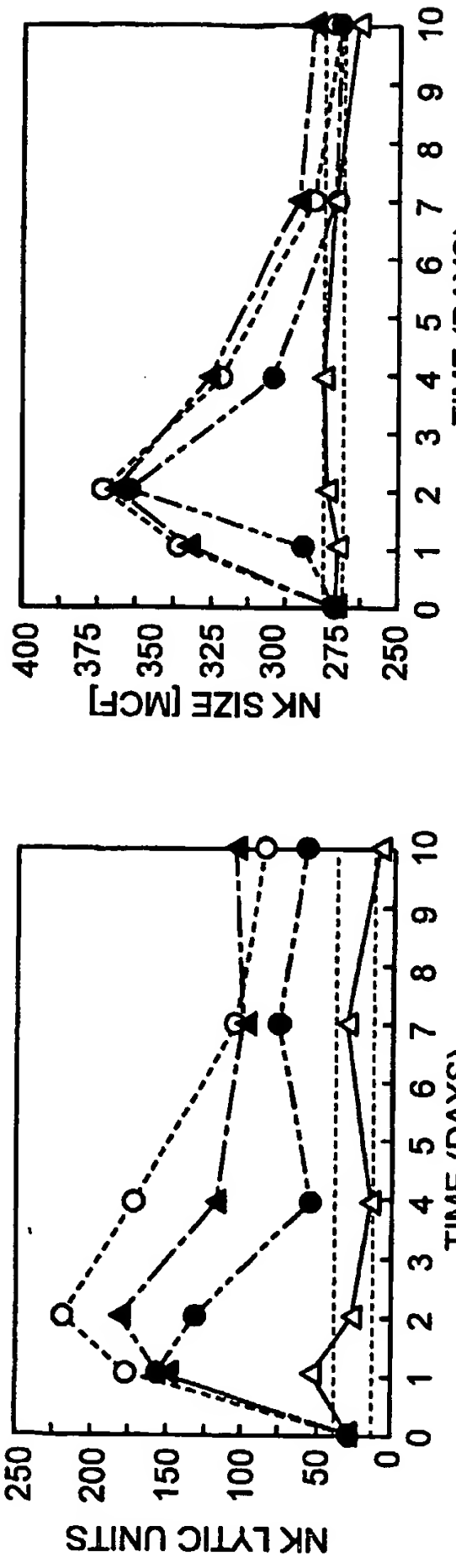


FIG. 1A

FIG. 1B

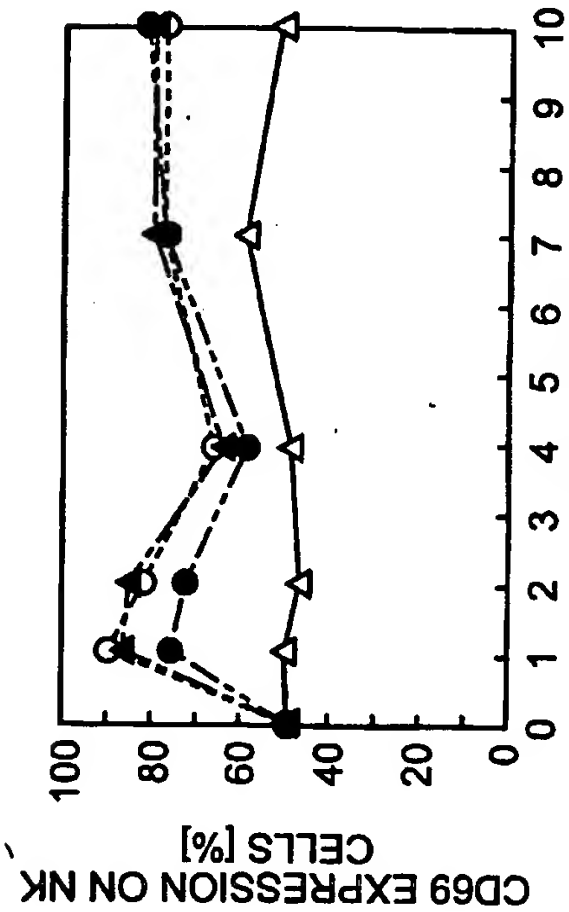


FIG. 1C

FIG. 1D

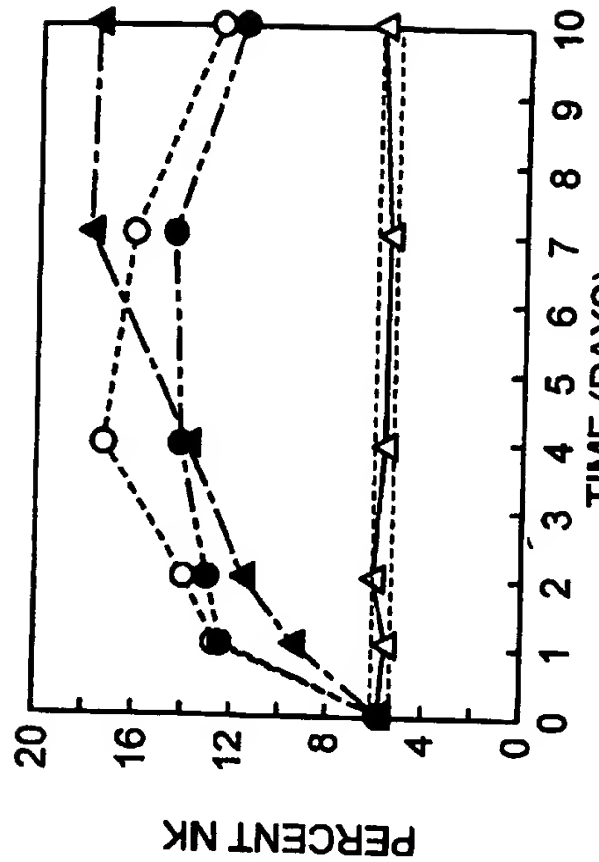


FIG. 1F

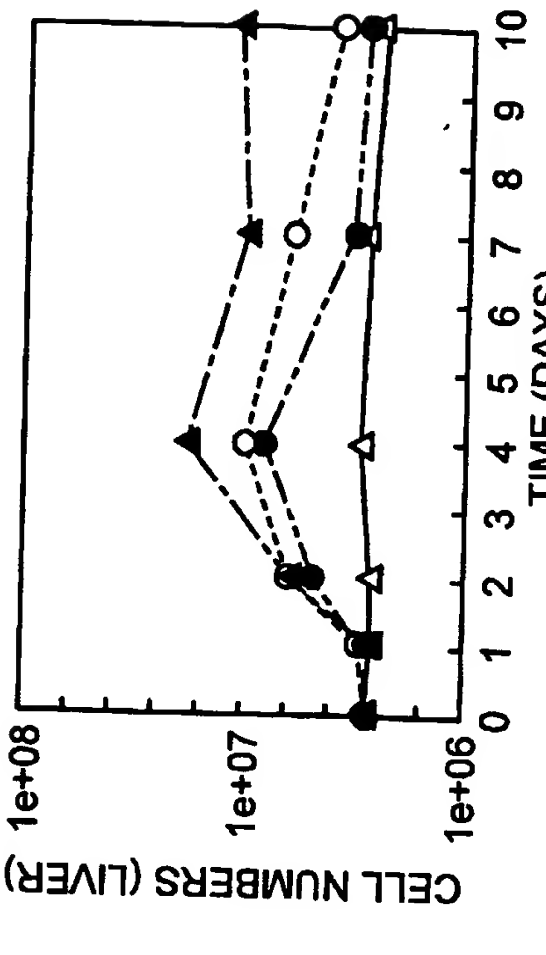


FIG. 1H

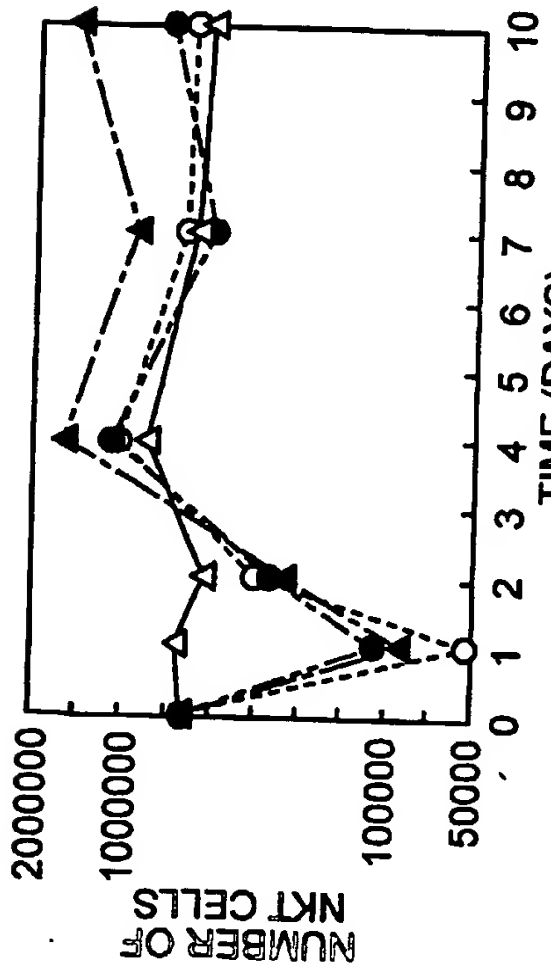


FIG. 1E

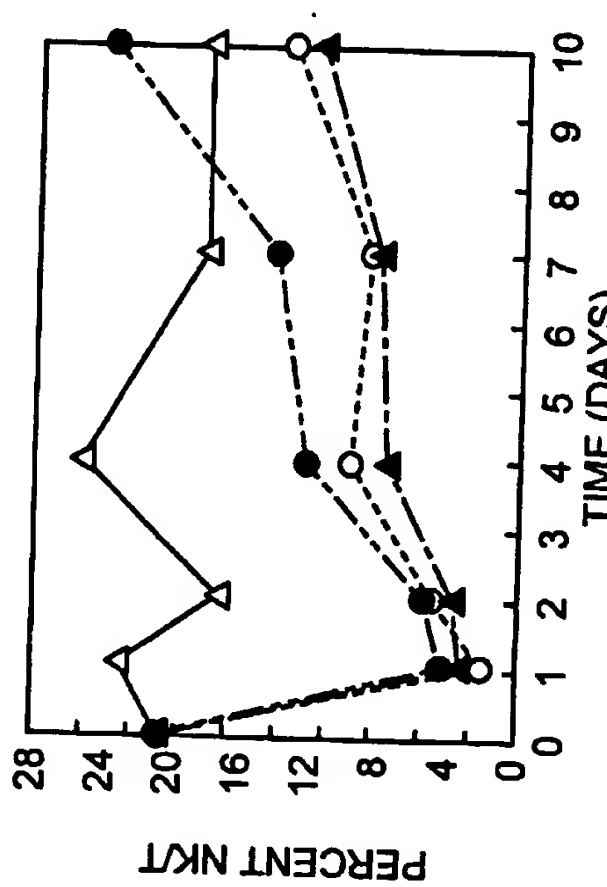


FIG. 1G

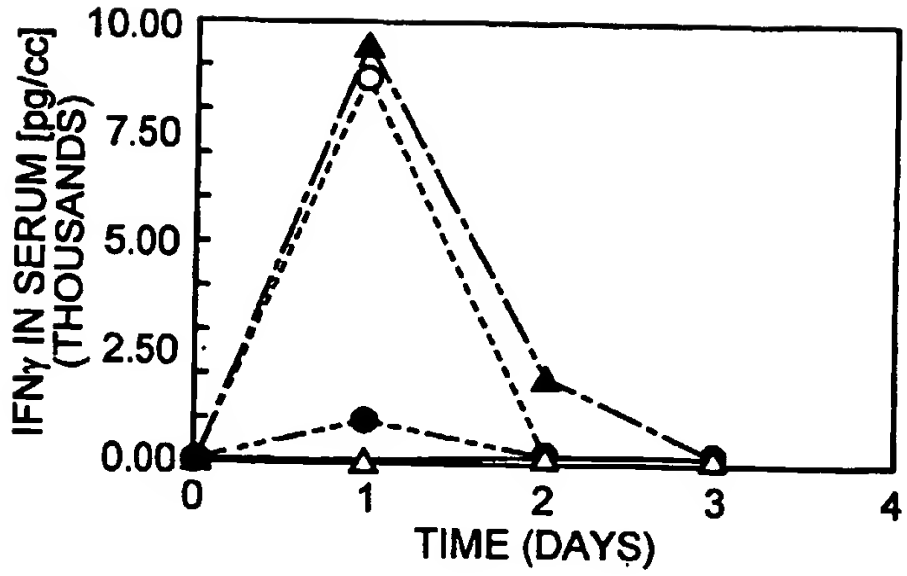

IFNγ IN SERUM [pg/cc]
(THOUSANDS)
10.00
7.50
5.00
2.50
0.00
0
1
2
3
4
TIME (DAYS)

FIG. 1I

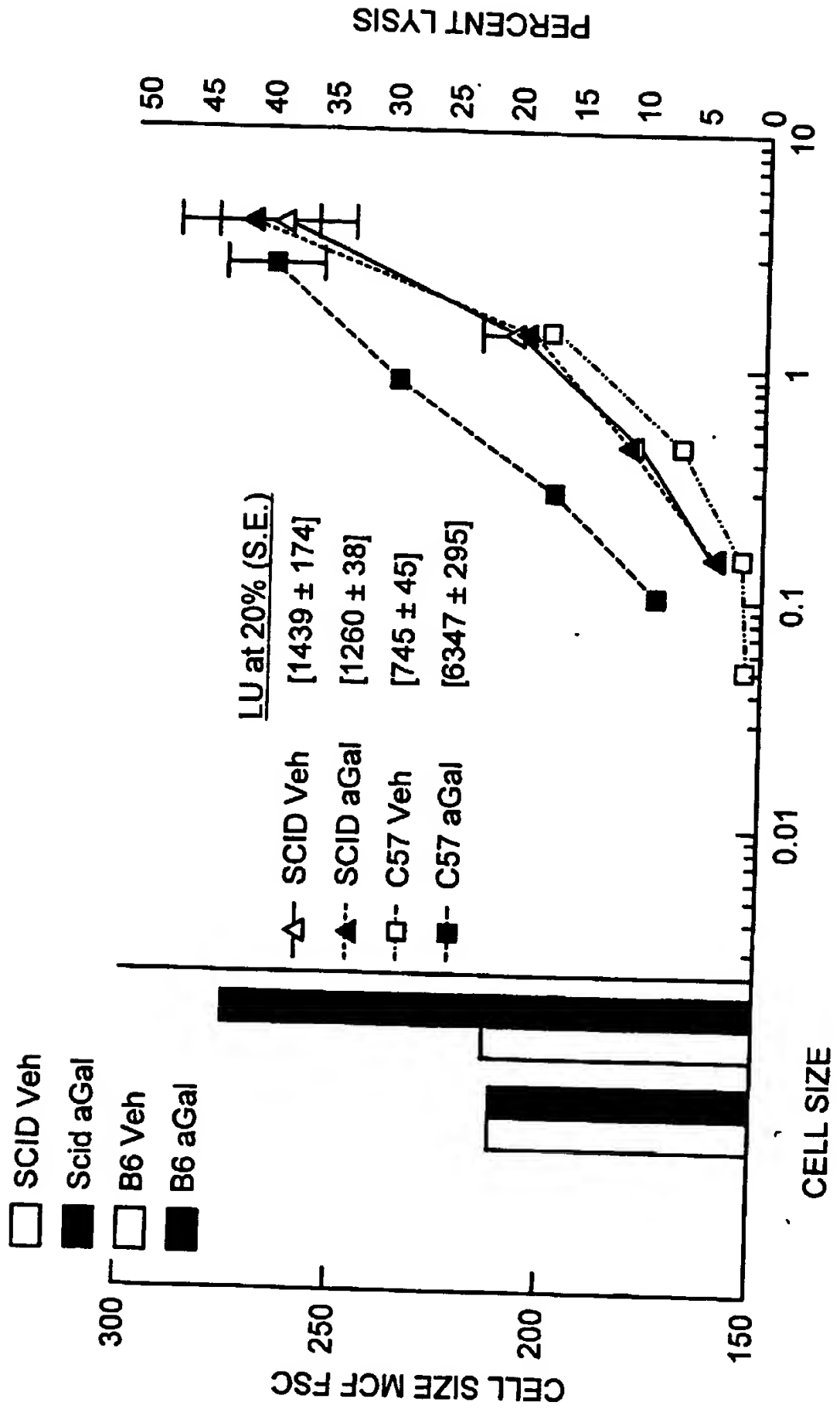

PERCENT LYSIS
50
45
40
35
30
25
20
15
10
5
0
10
1
0.1
0.01
NK ADJUSTED E:T RATIO
LU at 20% (S.E.)
[1439 ± 174]
[1260 ± 38]
[745 ± 45]
[6347 ± 295]
SCID Veh
SCID aGal
C57 Veh
C57 aGal
SCID Veh
Scid aGal
B6 Veh
B6 aGal
300
250
200
150
CELL SIZE MCF FSC
CELL SIZE

FIG. 2

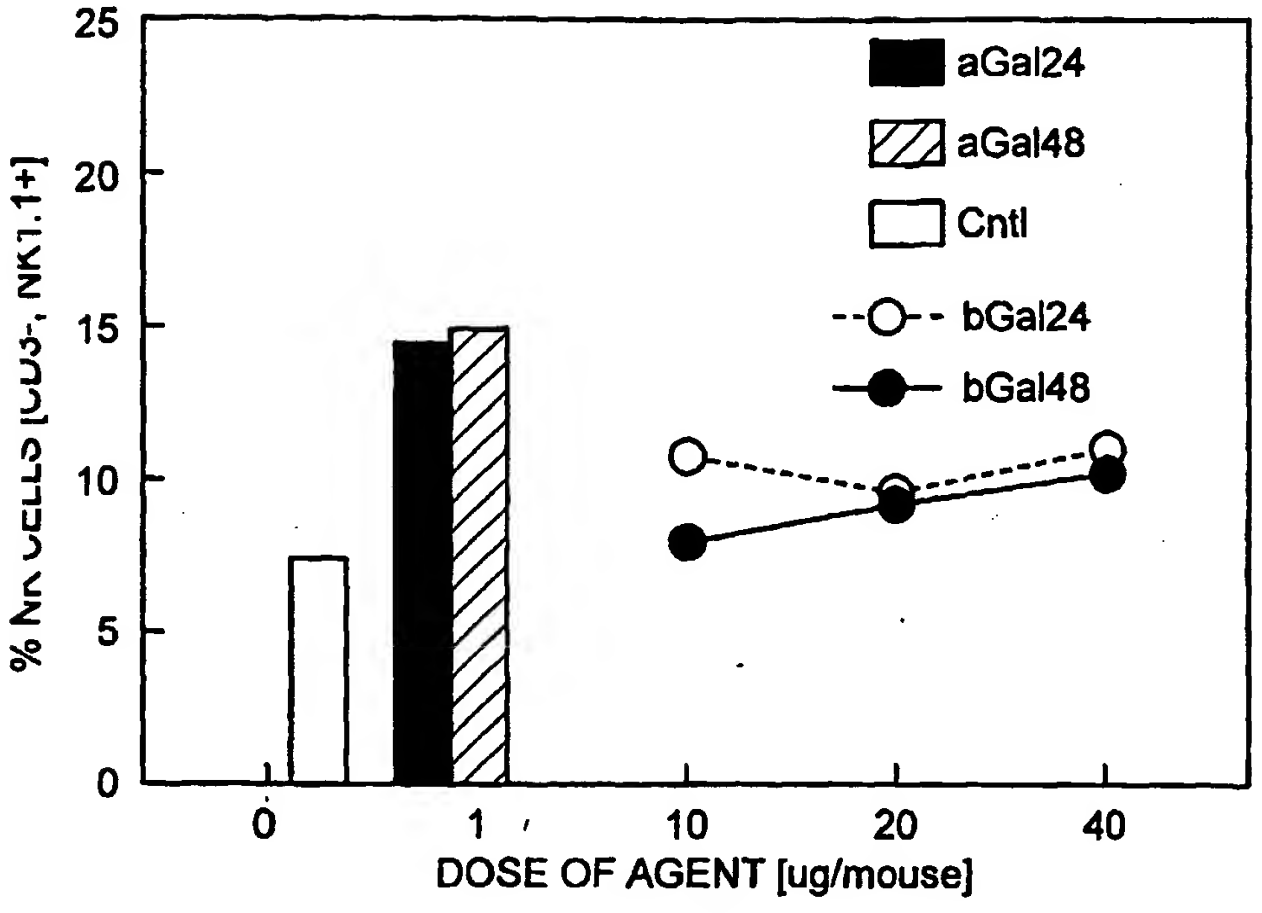

25
20
15
10
5
0
% NK CELLS [CD3-, NK1.1+]
aGal24
aGal48
Cntl
bGal24
bGal48
0
1
10
20
40
DOSE OF AGENT [ug/mouse]

FIG. 3A

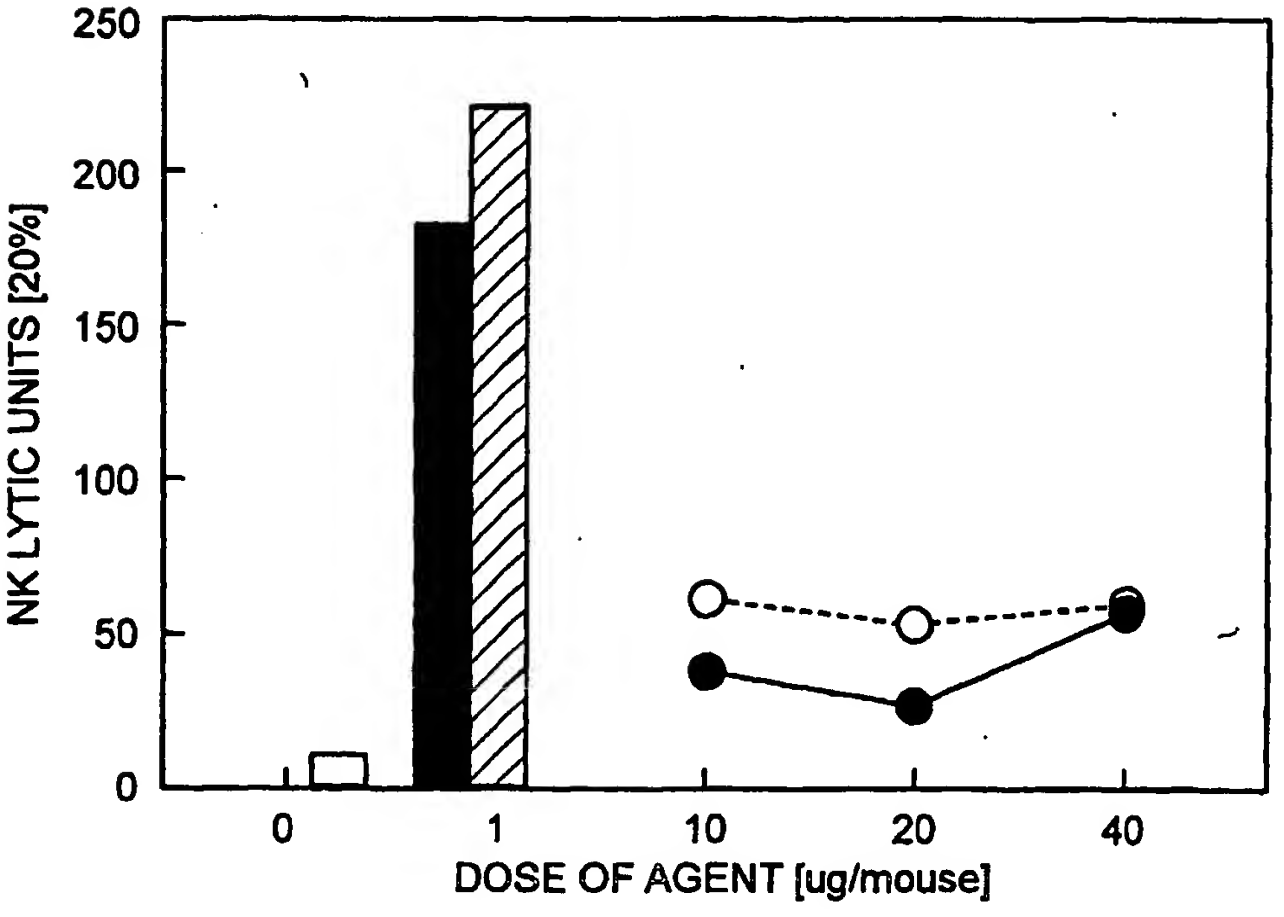

250
200
150
100
50
0
NK LYTIC UNITS [20%]
0
1
10
20
40
DOSE OF AGENT [ug/mouse]

FIG. 3B

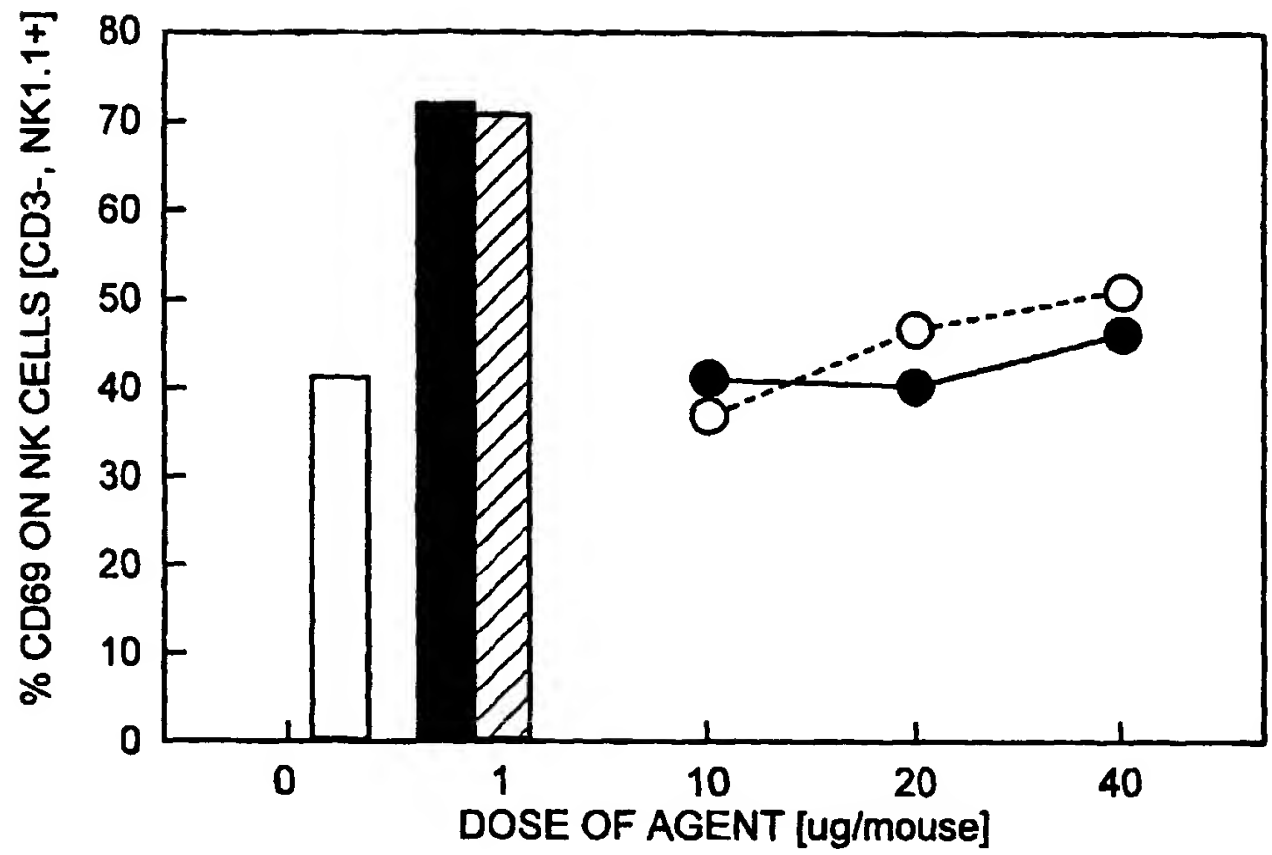

% CD69 ON NK CELLS [CD3-, NK1.1+]
80
70
60
50
40
30
20
10
0
0
1
10
20
40
DOSE OF AGENT [ug/mouse]

FIG. 3C

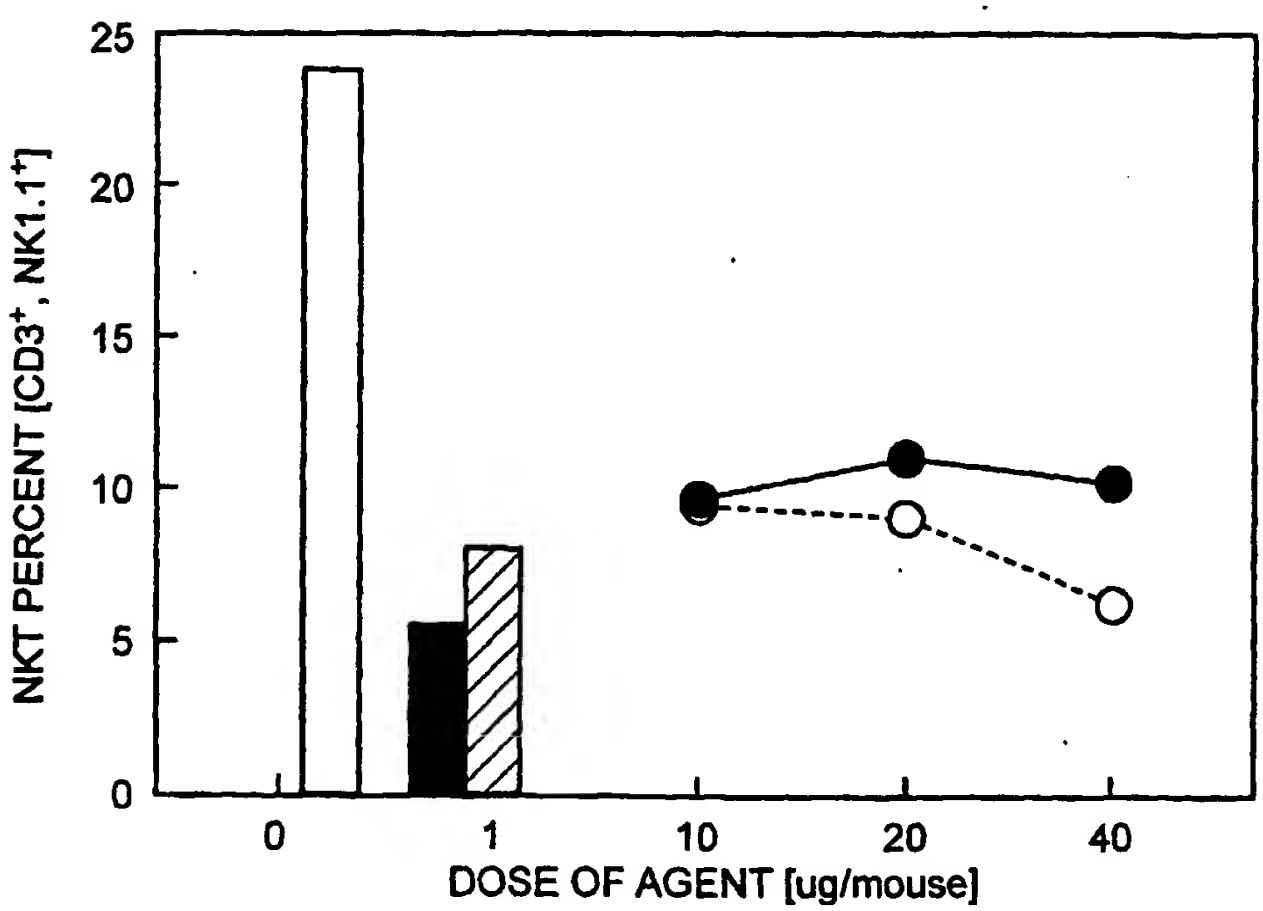
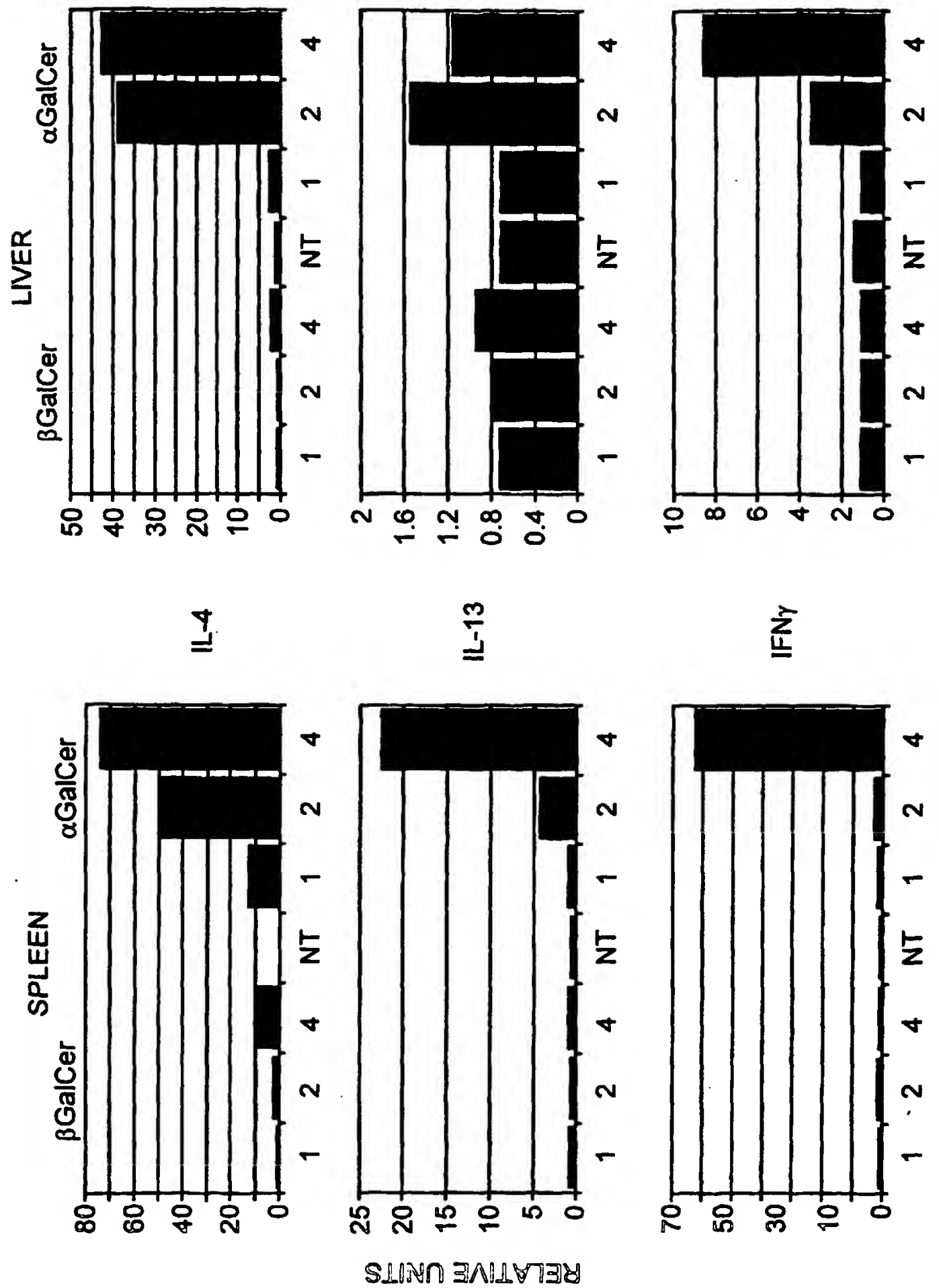

NKT PERCENT [CD3+, NK1.1+]
25
20
15
10
5
0
0
1
10
20
40
DOSE OF AGENT [ug/mouse]

FIG. 3D

FIG. 4


LIVER
βGalCer
αGalCer
SPLEEN
βGalCer
αGalCer
IL-4
IL-13
IFNγ
RELATIVE UNITS
1 2 4 NT 1 2 4
80 60 40 20 0
50 40 30 20 10 0
25 20 15 10 5 0
2 1.6 1.2 0.8 0.4 0
70 50 30 10 0
10 8 6 4 2 0

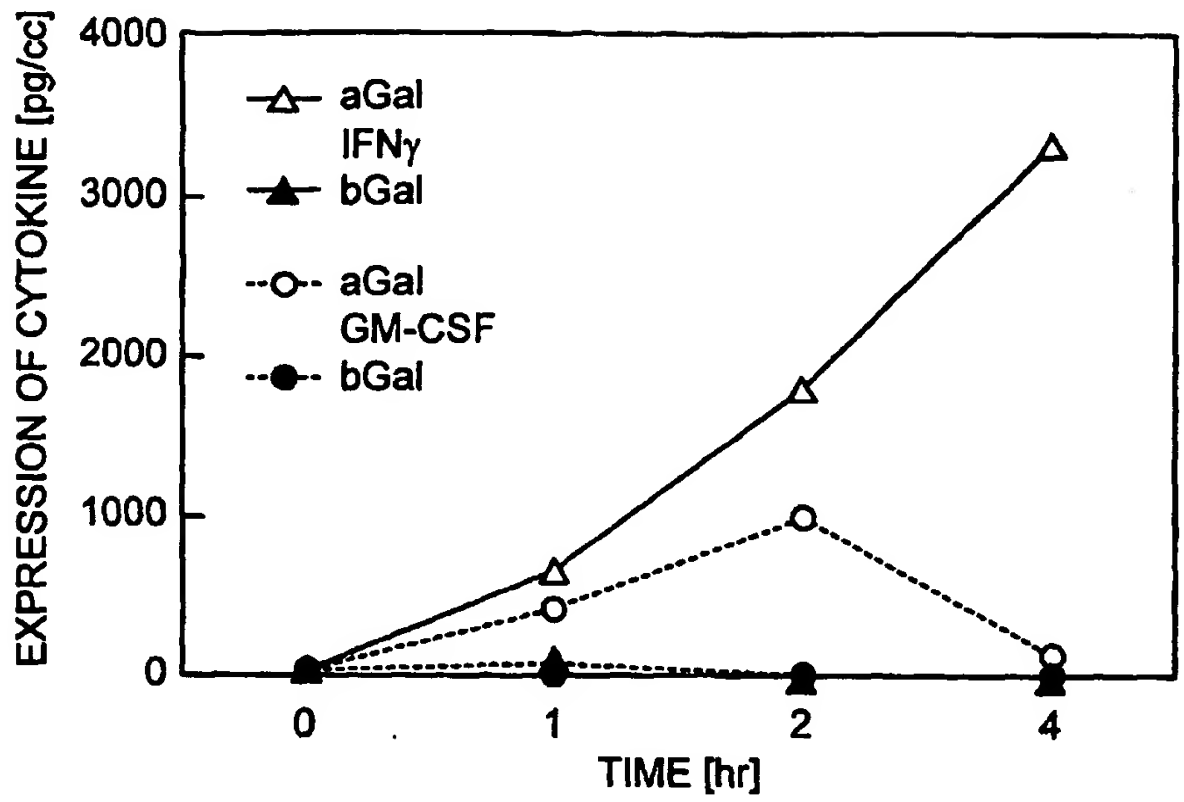

EXPRESSION OF CYTOKINE [pg/cc]
4000
3000
2000
1000
0
aGal
IFNγ
bGal
aGal
GM-CSF
bGal
0
1
2
4
TIME [hr]

FIG. 5A

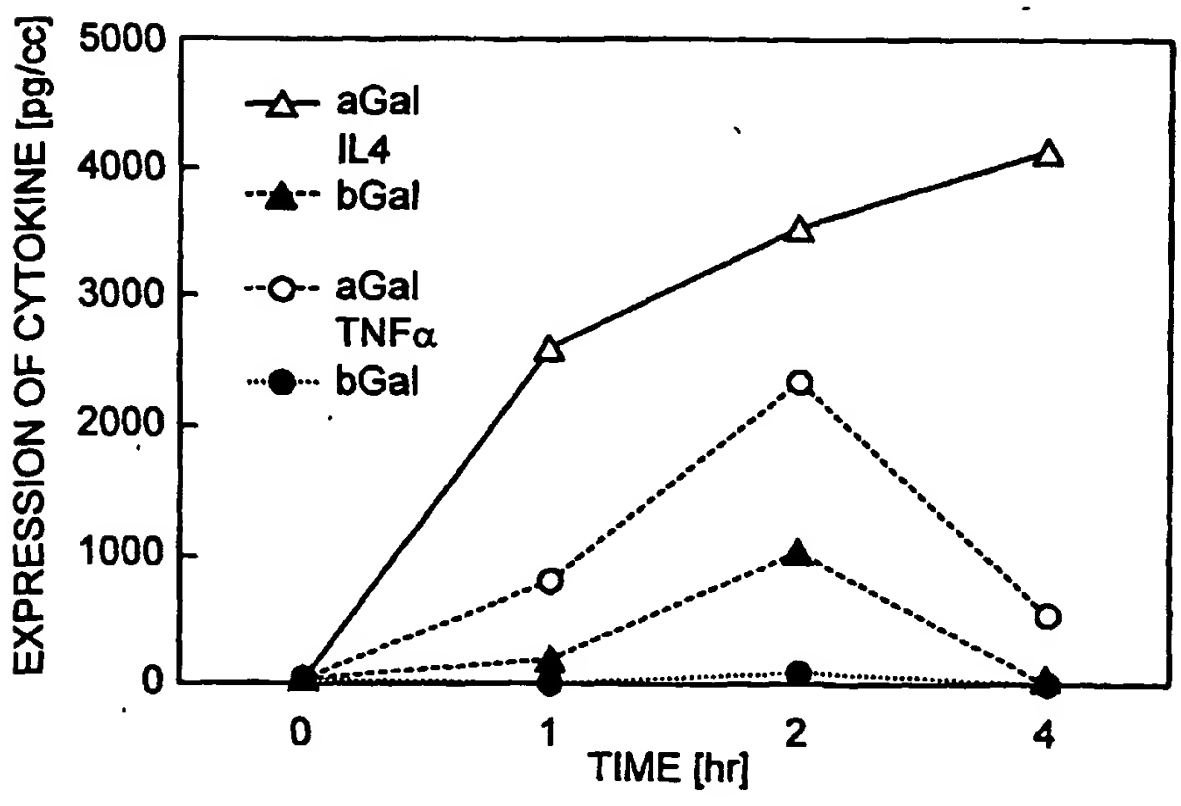

EXPRESSION OF CYTOKINE [pg/cc]
5000
4000
3000
2000
1000
0
aGal
IL4
bGal
aGal
TNFα
bGal
0
1
2
4
TIME [hr]

FIG. 5B

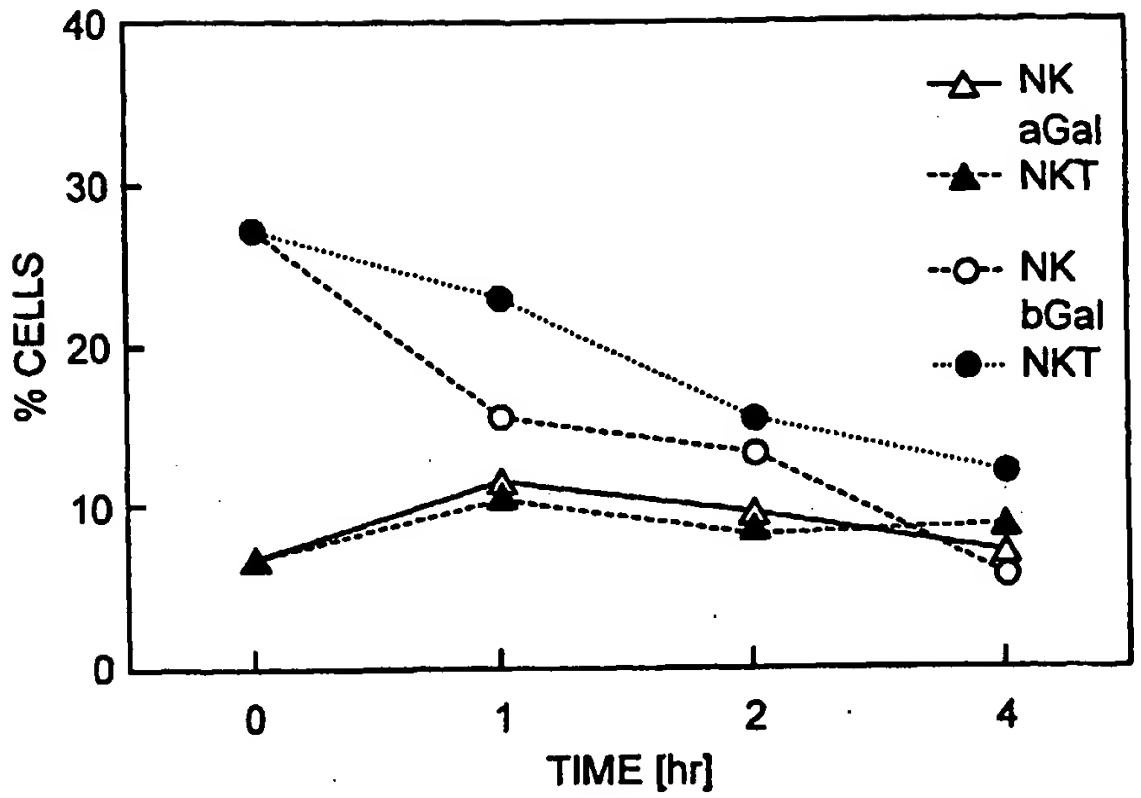

40
30
20
10
0
% CELLS
0
1
2
4
TIME [hr]
NK aGal
NKT
NK bGal
NKT

FIG. 5C

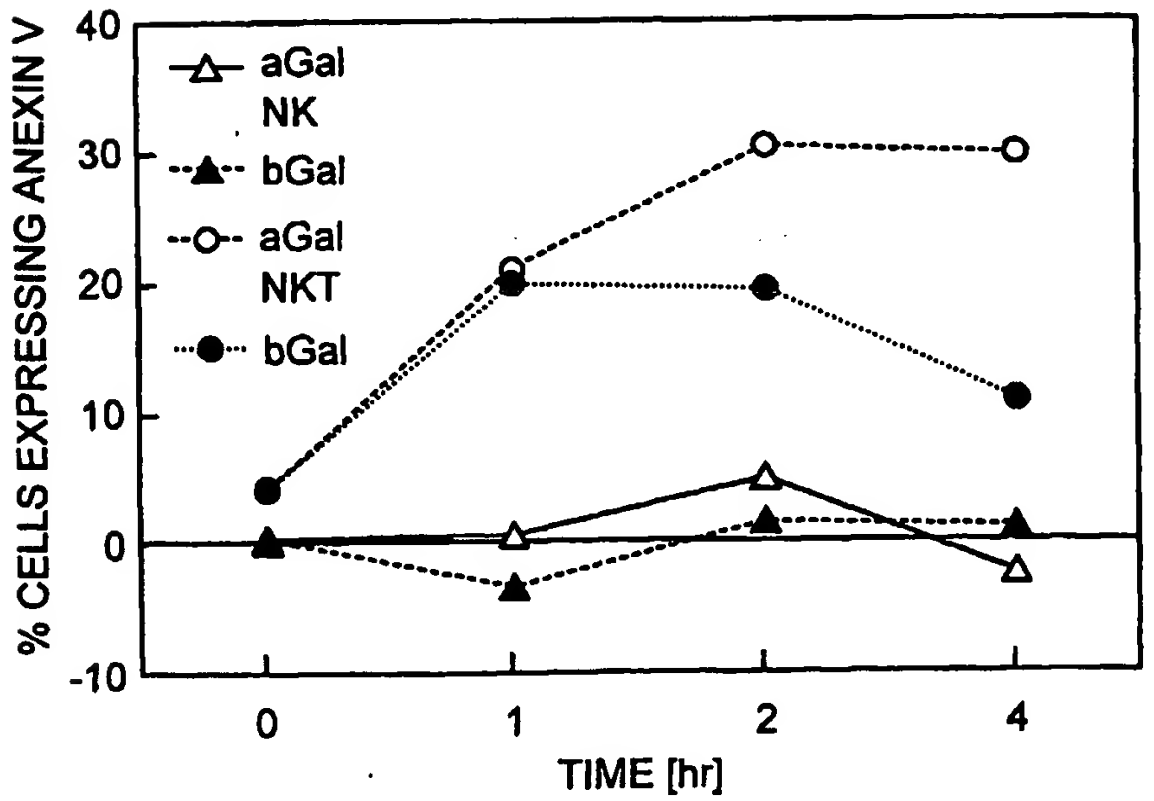

40
30
20
10
0
-10
% CELLS EXPRESSING ANEXIN V
0
1
2
4
TIME [hr]
aGal NK
bGal
aGal NKT
bGal

FIG. 5D

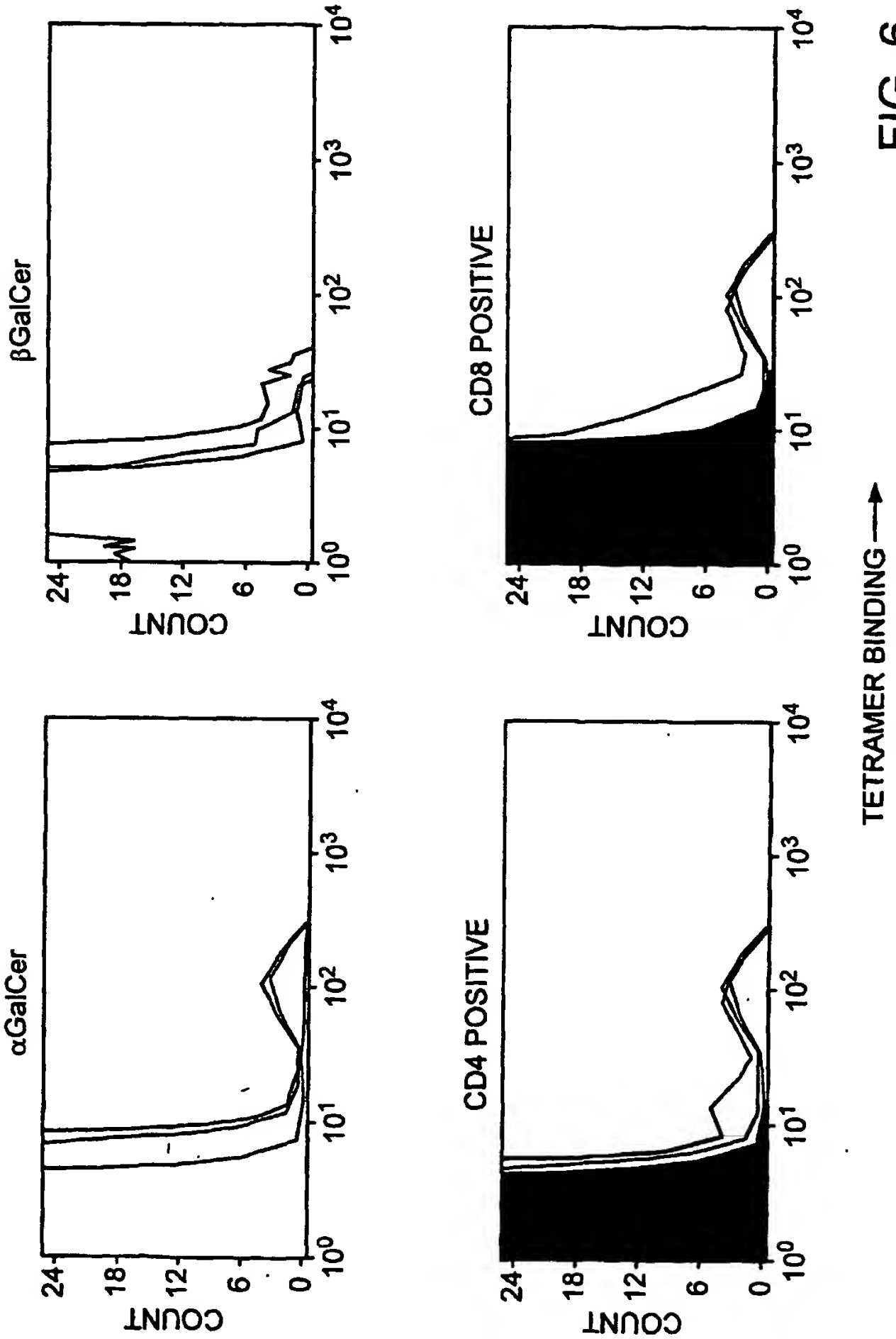

βGalCer
αGalCer
CD8 POSITIVE
CD4 POSITIVE
COUNT
TETRAMER BINDING
0
6
12
18
24
10^0
10^1
10^2
10^3
10^4

FIG. 6

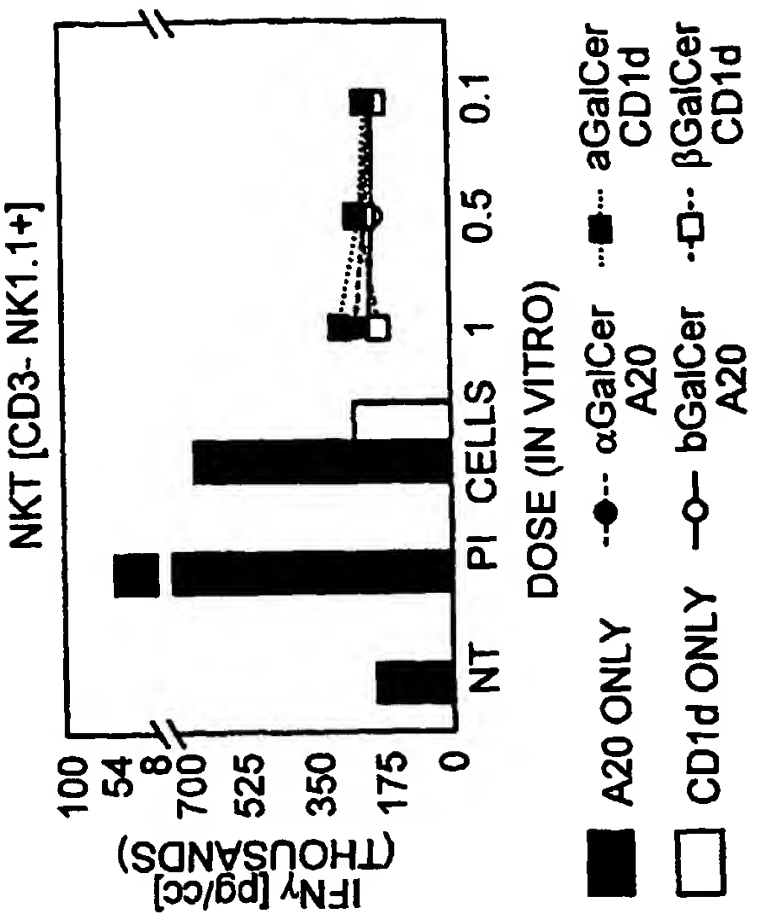

NKT [CD3- NK1.1+]
IFNγ [pg/cc] (THOUSANDS)
100
54
8
700
525
350
175
0
NT
PI
CELLS
1
0.5
0.1
DOSE (IN VITRO)
αGalCer A20
aGalCer CD1d
bGalCer A20
βGalCer CD1d
A20 ONLY
CD1d ONLY

FIG. 7B

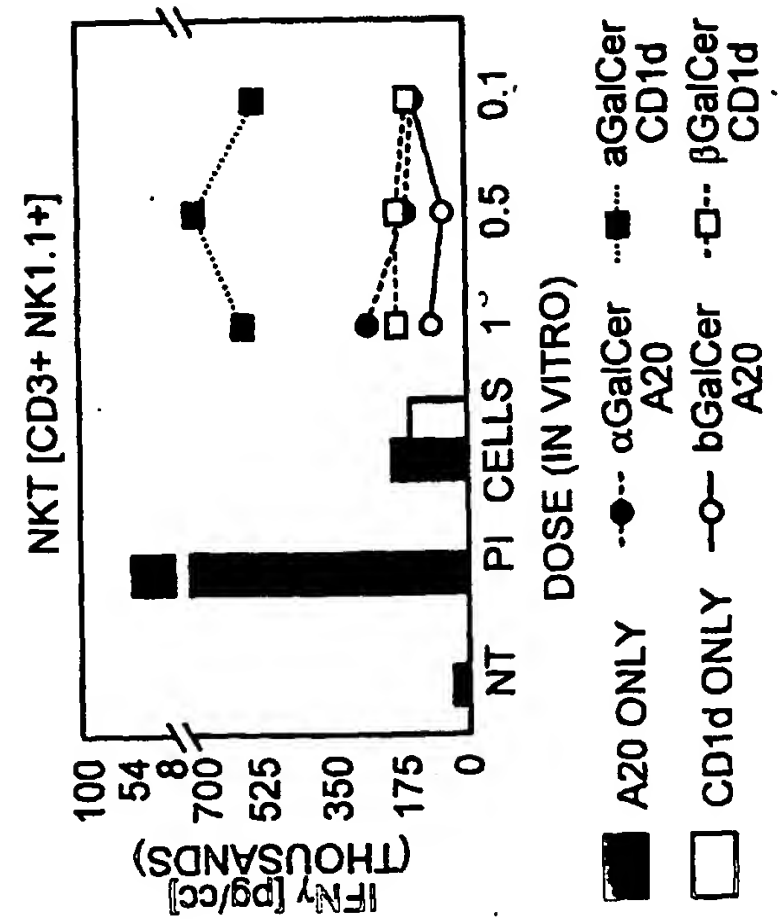

NKT [CD3+ NK1.1+]
IFNγ [pg/cc] (THOUSANDS)
100
54
8
700
525
350
175
0
NT
PI
CELLS
1
0.5
0.1
DOSE (IN VITRO)
αGalCer A20
aGalCer CD1d
bGalCer A20
βGalCer CD1d
A20 ONLY
CD1d ONLY

FIG. 7A

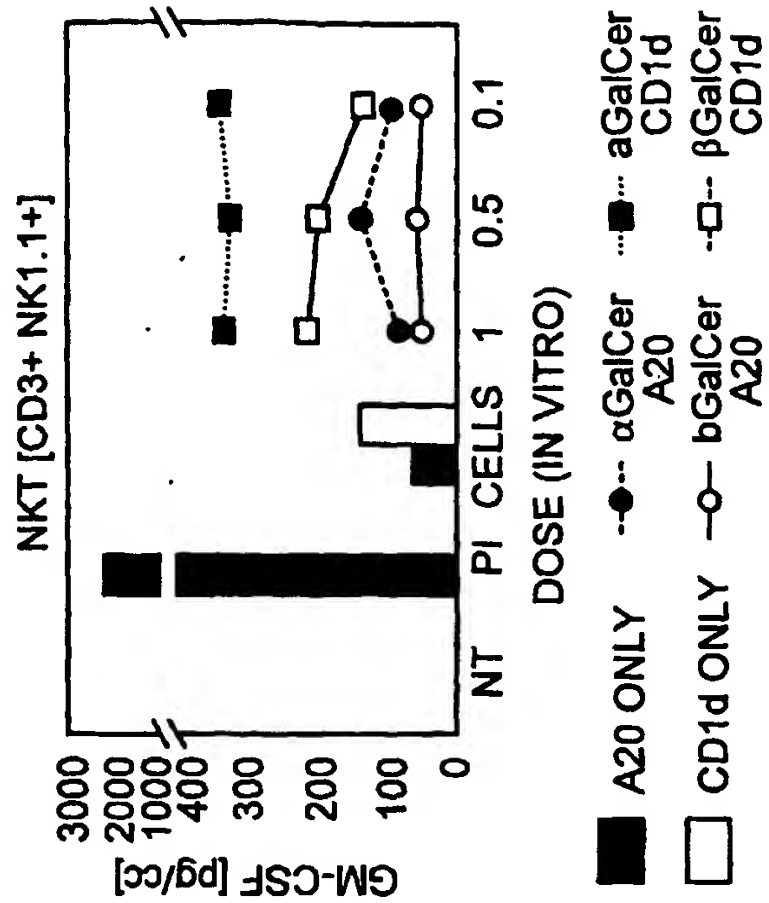

NKT [CD3+ NK1.1+]
GM-CSF [pg/cc]
3000
2000
1000
400
300
200
100
0
NT
PI CELLS
1
0.5
0.1
DOSE (IN VITRO)
A20 ONLY
CD1d ONLY
αGalCer A20
bGalCer A20
aGalCer CD1d
βGalCer CD1d

FIG. 7D

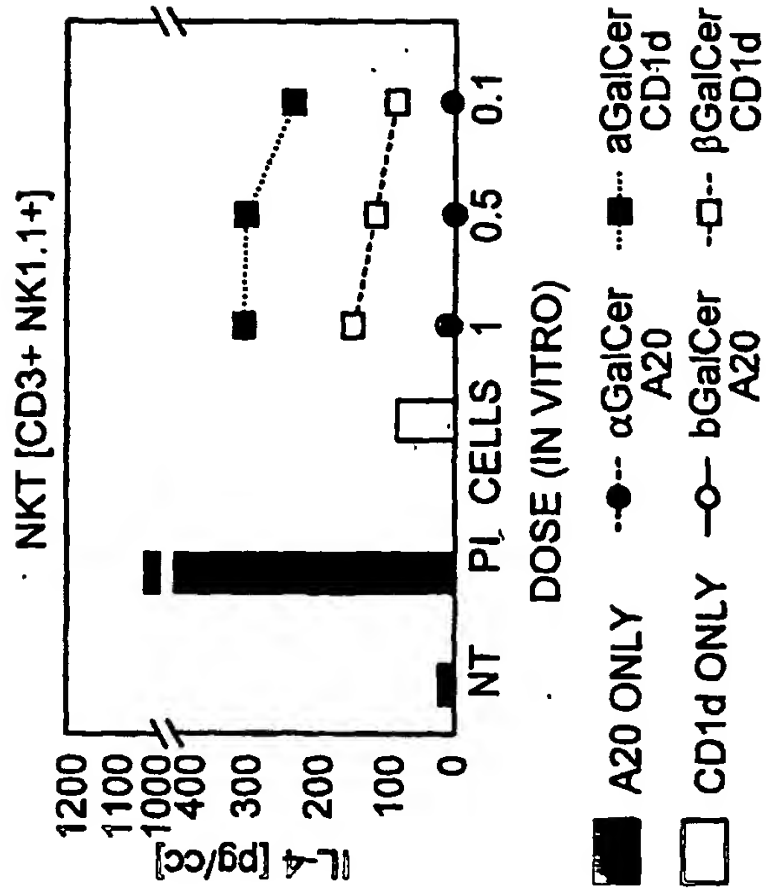

NKT [CD3+ NK1.1+]
IL-4 [pg/cc]
1200
1100
1000
400
300
200
100
0
NT
PI CELLS
1
0.5
0.1
DOSE (IN VITRO)
A20 ONLY
CD1d ONLY
αGalCer A20
bGalCer A20
aGalCer CD1d
βGalCer CD1d

FIG. 7C

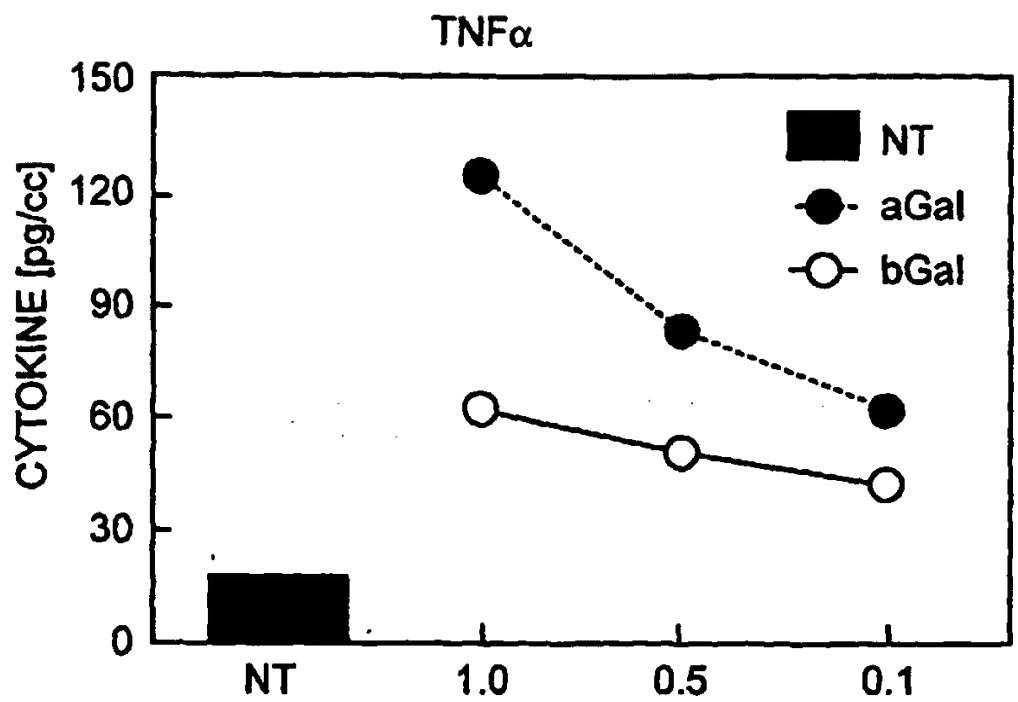

TNFα
CYTOKINE [pg/cc]
150
120
90
60
30
0
NT
aGal
bGal
NT
1.0
0.5
0.1

FIG. 8A

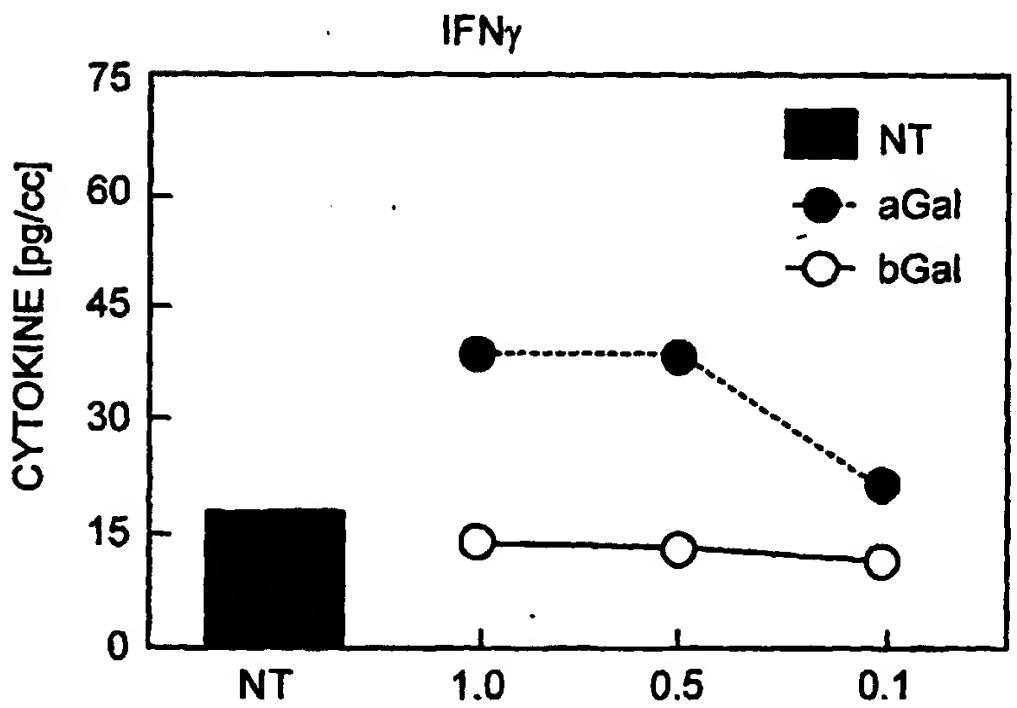

IFNγ
CYTOKINE [pg/cc]
75
60
45
30
15
0
NT
aGal
bGal
NT
1.0
0.5
0.1

FIG. 8B

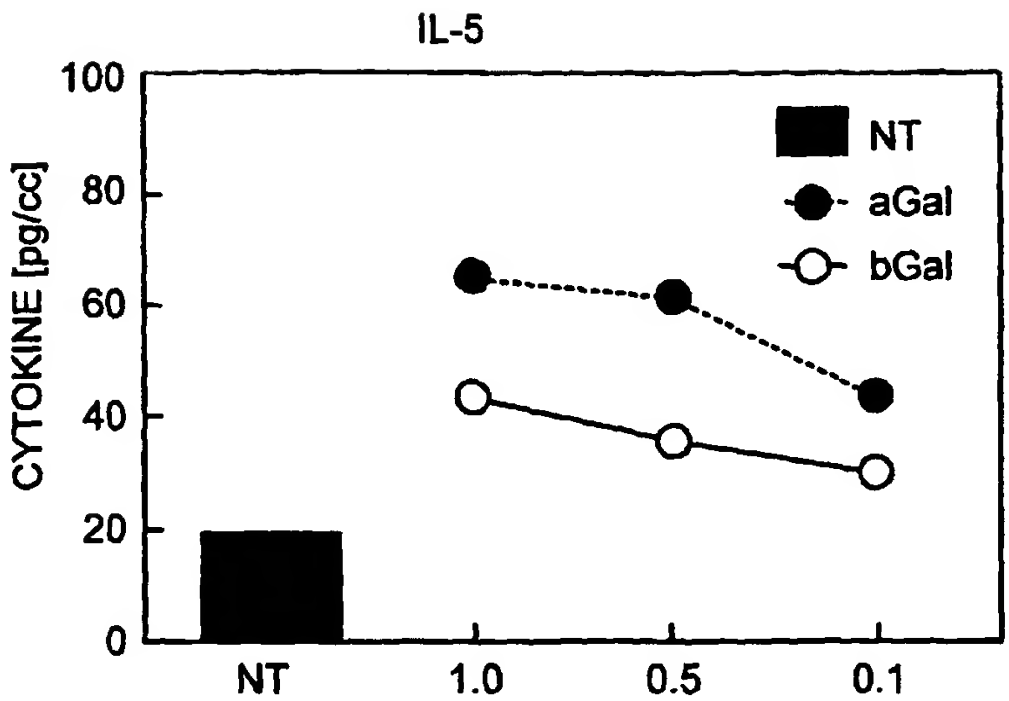

IL-5
CYTOKINE [pg/cc]
100
80
60
40
20
0
NT
1.0
0.5
0.1
NT
aGal
bGal

FIG. 8C

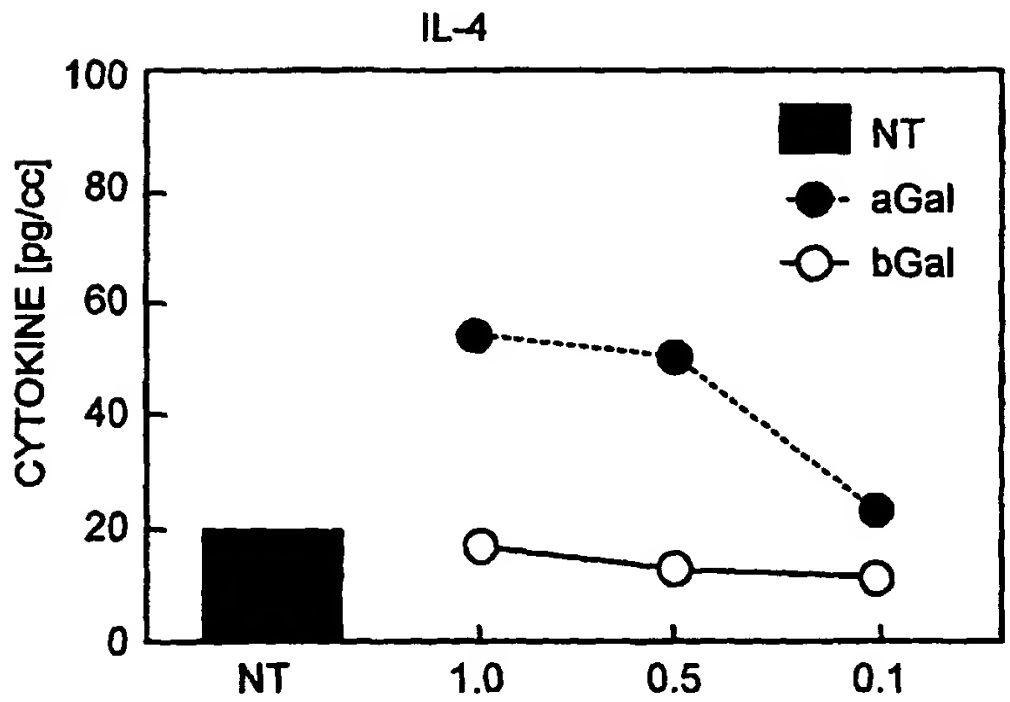

IL-4
CYTOKINE [pg/cc]
100
80
60
40
20
0
NT
1.0
0.5
0.1
NT
aGal
bGal

FIG. 8D

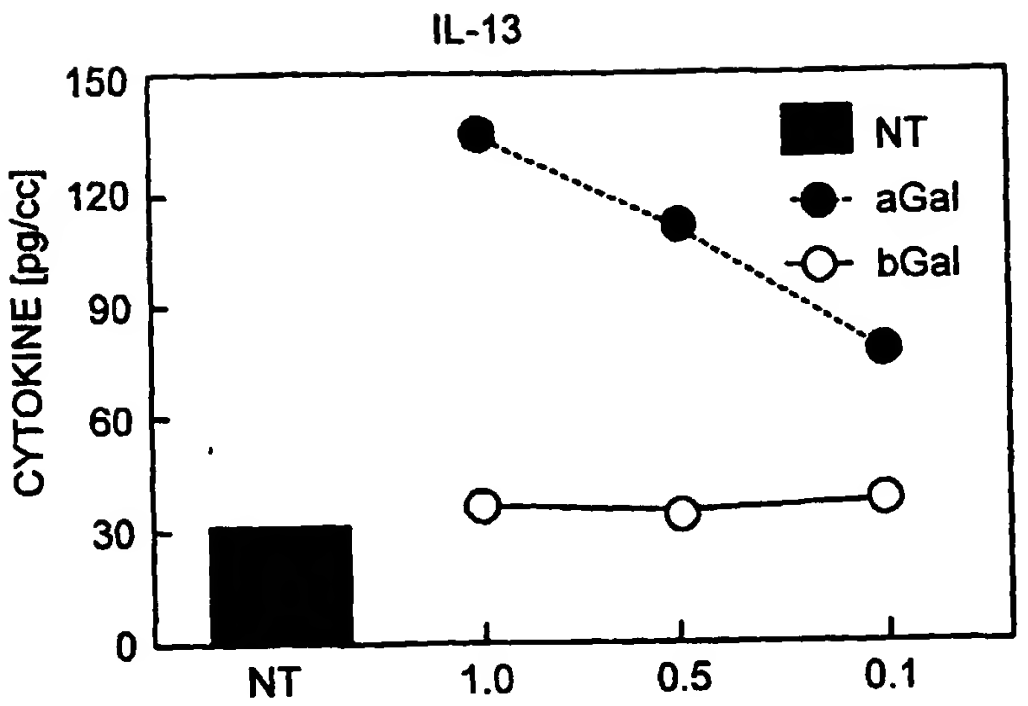

IL-13
CYTOKINE [pg/cc]
150
120
90
60
30
0
NT
aGal
bGal
NT
1.0
0.5
0.1

FIG. 8E

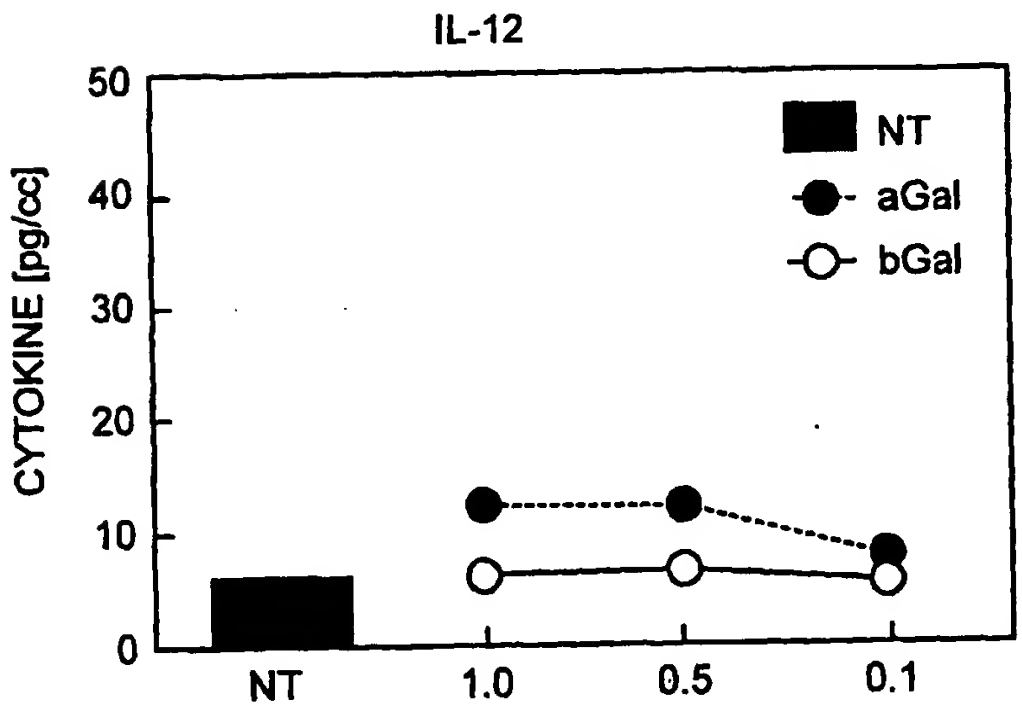

IL-12
CYTOKINE [pg/cc]
50
40
30
20
10
0
NT
aGal
bGal
NT
1.0
0.5
0.1

FIG. 8F

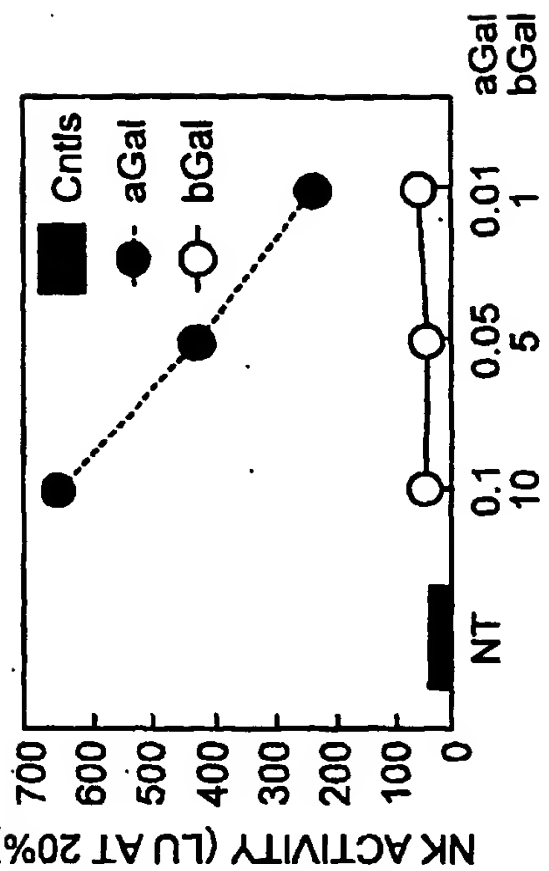

NK ACTIVITY (LU AT 20%)
700
600
500
400
300
200
100
0
Cntls
aGal
bGal
NT
0.1
10
0.05
5
0.01
1
aGal
bGal

FIG. 9B

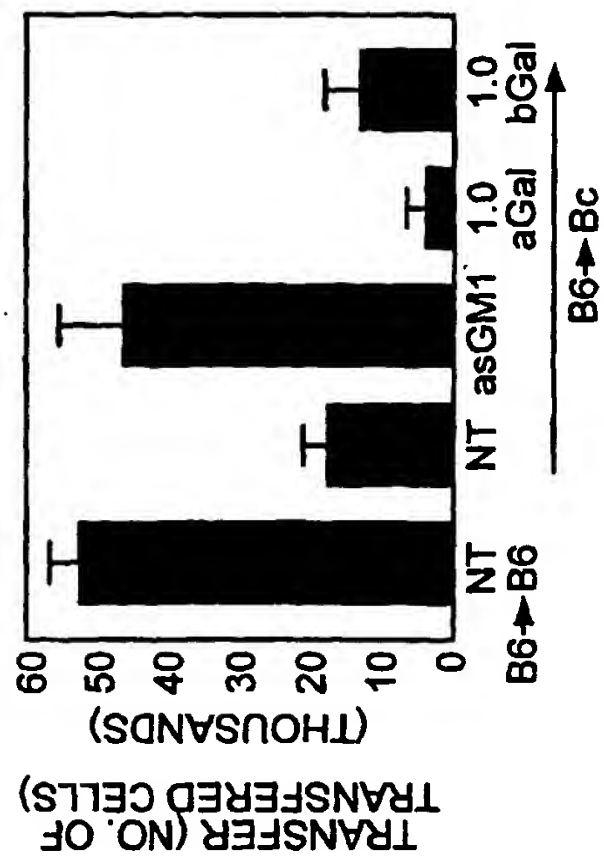

TRANSFER (NO. OF TRANSFERED CELLS)
(THOUSANDS)
60
50
40
30
20
10
0
B6→B6
NT
B6→Bc
NT
asGM1
1.0
aGal
1.0
bGal

FIG. 9A

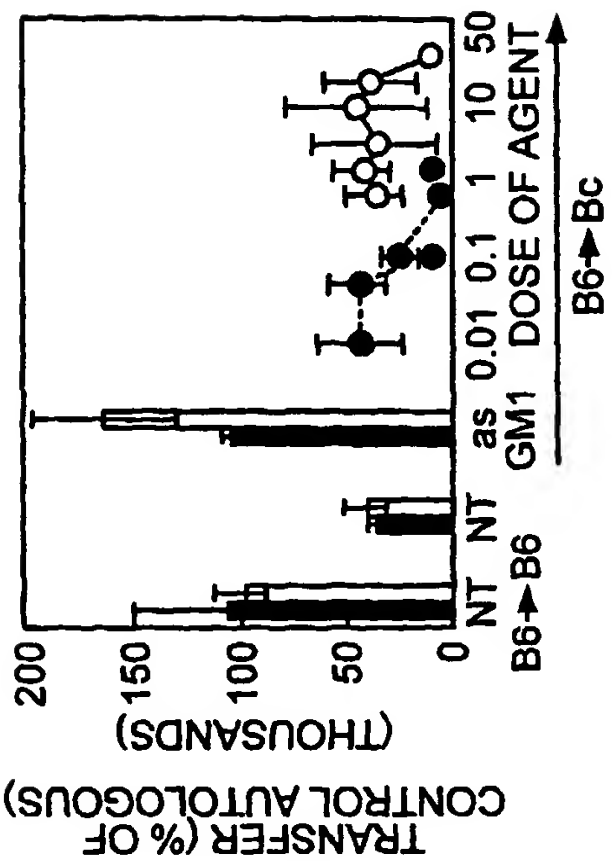

TRANSFER (% OF CONTROL AUTOLOGOUS)
(THOUSANDS)
200
150
100
50
0
B6→B6
NT
NT
as
GM1
0.01
0.1
1
10
50
DOSE OF AGENT
B6→Bc

FIG. 9D

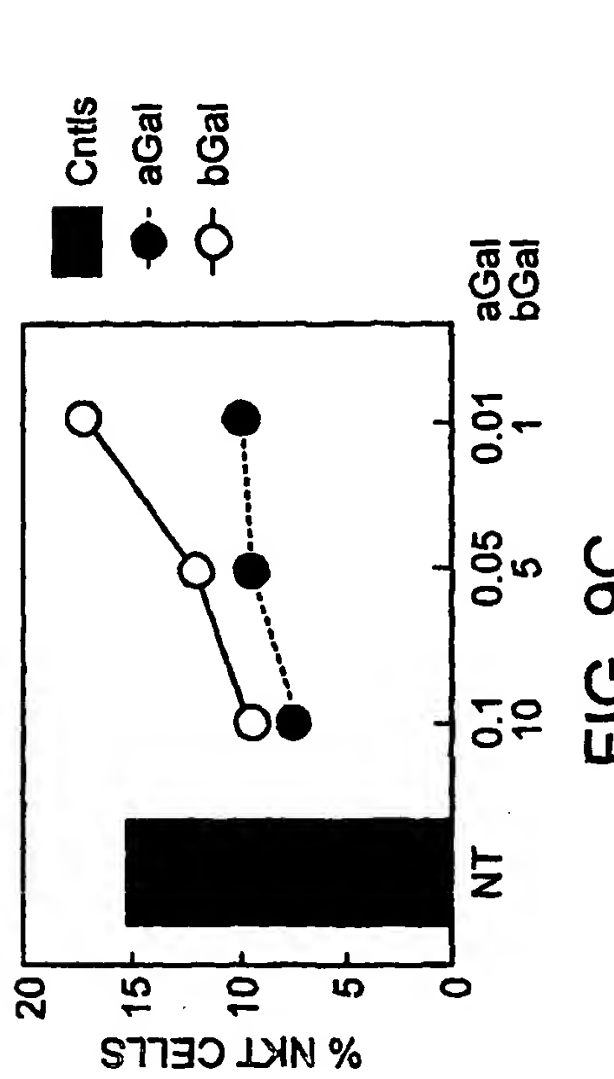

% NKT CELLS
20
15
10
5
0
NT
0.1
10
0.05
5
0.01
1
aGal
bGal
Cntls
aGal
bGal

FIG. 9C

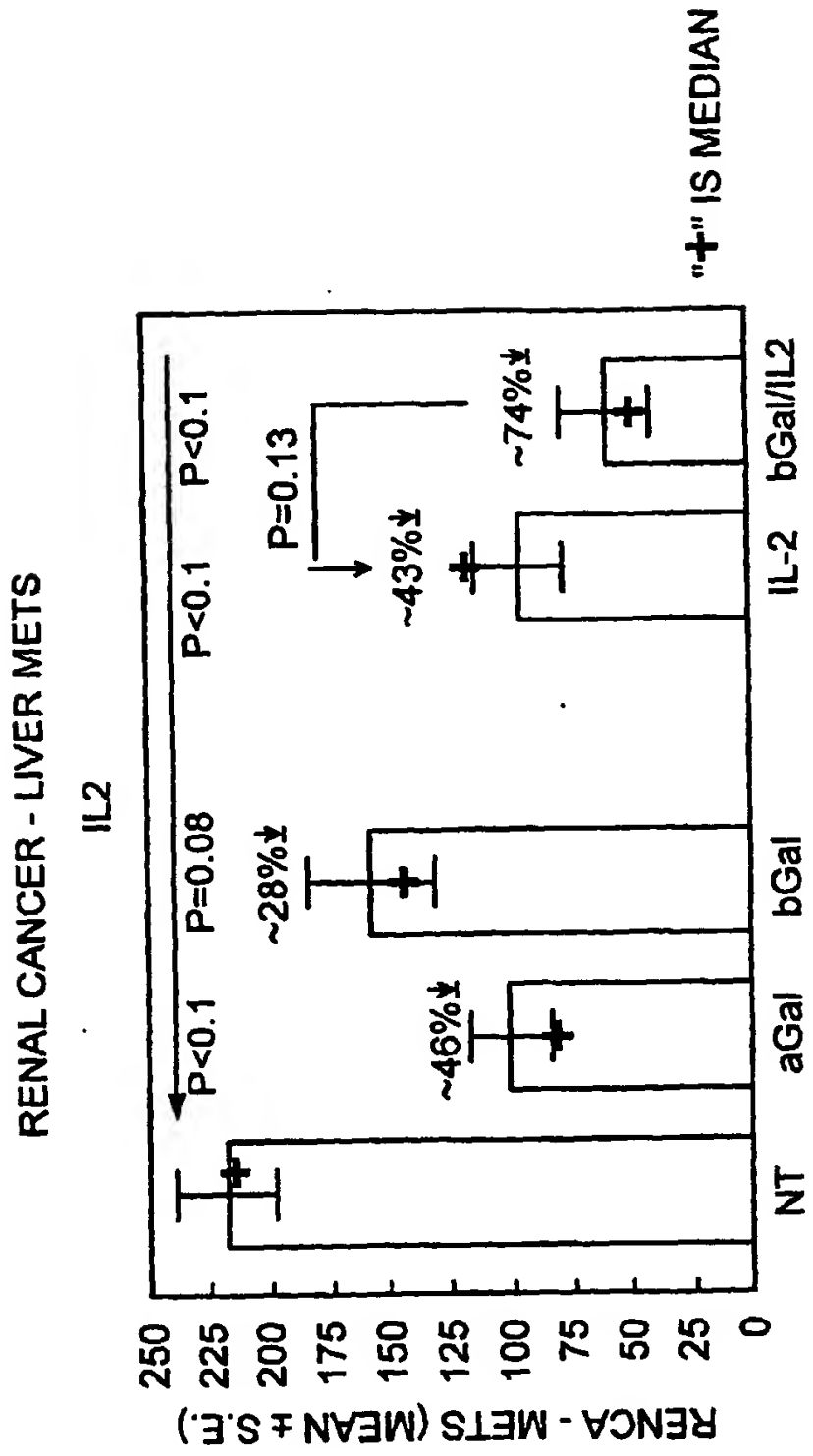

RENAL CANCER - LIVER METS
IL2
P<0.1
P=0.08
P<0.1
~28%↓
~46%↓
P<0.1
P=0.13
~43%↓
~74%↓
NT
aGal
bGal
IL-2
bGal/IL2
"+" IS MEDIAN
RENCA - METS (MEAN ± S.E.)
250
225
200
175
150
125
100
75
50
25
0

FIG. 10

```
   1 ctctcacagt tcctatcagg gcgtcctgtg actcgccttg ccacatctgt gtctctggaa
  61 cccaggacct ctggacctca ggttcccaag acttcagtcc tggtctgctc aggaattgaa
 121 gatgtaagga attgaagata ggagagatac cttgaaaaag tagagaacag tcatgaggca
 181 gctttcatca cacccttta acatttatct aaaagaattt aaattctttt tcaaaaatta
 241 cactacaagt ttataagccc aaatggctct gtgaaatcag aagtgcaaag gtgtgcaaac
 301 ttgtatctga agacctacca gggacaagca ggtaagagct gatgtgagtg tgtgtgatgg
 361 gatctgtaag gaactggaac acacatgtcc tatccaaagg aatcagctgc agctgcttgt
 421 tgtcaagtat aaagtcagga cctggcttgg ctttaaccgt ttttcaagaa aactggaaat
 481 ctggattttc agcgaacatg cctgatttta aaaggttgac tcaagttttt acaaaatact
 541 atgtgggaca cctcaaatac atacctactg actgatgaca aacccaggag tttgtgtgtc
 601 ttttataaaa agtttgccct ggatgtcata ttggcagttg gaggacacag tttctattgt
 661 aaatttggat ttacgactga agaaggacat tttctcttta aaagaaagtt aggttataag
 721 aaacagaggc gtctcacatt tttacttggt gtaattaata aa
```

FIG. 11

FIG. 12

MGCLLFLLLWALLQAWGSAEVPQRLFPLRCLQISSFANSSWTRTDGLAWLGEL
QTHSWSNDSDTVRSLKPWSQGTFSDQQWETLQHIFRVYRSSFTRDVKEFAK
MLRLSYPLELQVSAGCEVHPGNASNNFFHVAFQGKDILSFQGTSWEPTQEAPL
WVNLAIQVLNQDKWTRETVQWLLNGTCPQFVSGLLESGKSELKKQVKPKAWL
SRGPSPGPGRLLLVCHVSGFYPKPVTVKWMRGEQEQQGTQPGDILPNADET
WYLRATLDVVAGEAAGLSCRVKHSSLEGQDIVLYWGGSYTSMGLIALAVLACL
LFLLIVGFTSRFKRQTSYQGVL